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(54) **Method for producing L-lysine**

(57) A coryneform bacterium harboring an aspartokinase in which feedback inhibition by L-lysine and L-threonine is substantially desensitized, and comprising an enhanced DNA sequence coding for a dihydrodipicolinate reductase, an enhanced DNA sequence coding for dihydropicolinate reductase, an enhanced DNA sequence coding for dihydropicolinate synthase, an enhanced DNA sequence coding for diaminopimelate decarboxylase and an enhanced DNA sequence coding for aspartate aminotransferase; a method for producing L-lysine comprising the steps of cultivating the coryneform bacterium in an appropriate medium to allow L-lysine to be produced and accumulated in a culture of the bacterium, and collecting L-lysine from the culture; and a recombinant DNA usable for production of the coryneform bacterium.

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Description

BACKGROUND OF THE INVENTION

5 The present invention relates to a method for producing L-lysine by cultivating a microorganism obtained by modifying a coryneform bacterium used for fermentative production of amino acid or the like by means of a technique based on genetic engineering.

L-Lysine, which is used as a fodder additive, is usually produced by a fermentative method by using an L-lysine-producing mutant strain belonging to the coryneform bacteria. Various L-lysine-producing bacteria known at present are those created by artificial mutation starting from wild type strains belonging to the coryneform bacteria.

10 As for the coryneform bacteria, there are disclosed a vector plasmid which is autonomously replicable in bacterial cells and has a drug resistance marker gene (see United States Patent No. 4, 514, 502), and a method for introducing a gene into bacterial cells (for example, Japanese Patent Application Laid-open No. 2-207791). There is also disclosed a possibility for breeding an L-threonine- or L-isoleucine-producing bacterium by using the techniques as described above (see United States Patent Nos. 4,452,890 and 4,442,208). As for breeding of an L-lysine-producing bacterium, a technique is known, in which a gene participating in L-lysine biosynthesis is incorporated into a vector plasmid to amplify the gene in bacterial cells (for example, Japanese Patent Application Laid-open No. 56-160997).

15 Known genes for L-lysine biosynthesis include, for example, a dihydrodipicolinate reductase gene (Japanese Patent Application Laid-open No. 7-75578) and an aspartate aminotransferase gene (Japanese Patent Application Laid-open No. 6-102028) in which a gene participating in L-lysine biosynthesis is cloned, as well as a phosphoenolpyruvate carboxylase gene (Japanese Patent Application Laid-open No. 60-87788), a dihydrodipicolinate synthase gene (Japanese Patent Publication No. 6-55149), and a diaminopimelate decarboxylase gene (Japanese Patent Application Laid-open No. 60-62994) in which amplification of a gene affects L-lysine productivity.

20 As for enzymes participating in L-lysine biosynthesis, a case is known for an enzyme which undergoes feedback inhibition when used as a wild type. In this case, L-lysine productivity is improved by introducing an enzyme gene having such mutation that the feedback inhibition is desensitized. Those known as such a gene specifically include, for example, an aspartokinase gene (International Publication Pamphlet of WO 94/25605).

25 As described above, certain successful results have been obtained by means of amplification of genes for the L-lysine biosynthesis system, or introduction of mutant genes. For example, a coryneform bacterium, which harbors a mutant aspartokinase gene with desensitized concerted inhibition by lysine and threonine, produces a considerable amount of L-lysine (about 25 g/L). However, this bacterium suffers decrease in growth speed as compared with a bacterium harboring no mutant aspartokinase gene. It is also reported that L-lysine productivity is improved by further introducing a dihydrodipicolinate synthase gene in addition to a mutant aspartokinase gene (Applied and Environmental Microbiology, 57 (6), 1746-1752 (1991)). However, such a bacterium suffers further decrease in growth speed.

30 As for the dihydrodipicolinate reductase gene, it has been demonstrated that the activity of dihydrodipicolinate reductase is increased in a coryneform bacterium into which the gene has been introduced, however, no report is included for the influence on L-lysine productivity (Japanese Patent Application Laid-open No. 7-75578).

35 In the present circumstances, no case is known for the coryneform bacteria, in which anyone has succeeded in remarkable improvement in L-lysine yield without restraining growth by combining a plurality of genes for L-lysine biosynthesis. No case has been reported in which growth is intended to be improved by enhancing a gene for L-lysine biosynthesis as well.

SUMMARY OF THE INVENTION

45 An object of the present invention is to improve the L-lysine productivity of a coryneform bacterium by using genetic materials of DNA sequences each coding for aspartokinase (hereinafter referred to as "AK", provided that a gene coding for an AK protein is hereinafter referred to as "lysC", if necessary), dihydrodipicolinate reductase (hereinafter referred to as "DDPR", provided that a gene coding for a DDPR protein is hereinafter referred to as "dapB", if necessary), dihydrodipicolinate synthase (hereinafter abbreviate as "DDPS", provided that a gene coding for a DDPS protein is hereinafter referred to as "dapA", if necessary), diaminopimelate decarboxylase (hereinafter referred to as "DDC", provided that a gene coding for a DDC protein is hereinafter referred to as "lysA", if necessary), and aspartate aminotransferase (hereinafter referred to as "AAT", provided that a gene coding for an AAT protein is hereinafter referred to as "aspC", if necessary) which are important enzymes for L-lysine biosynthesis in cells of coryneform bacteria.

50 The principle of the present invention is based on the fact that the L-lysine productivity can be improved by enhancing mutant lysC (hereinafter simply referred to as "mutant lysC", if necessary) coding for mutant AK (hereinafter simply referred to as "mutant AK", if necessary) in which concerted inhibition by lysine and threonine is desensitized, dapA, dapB, lysA and aspC in combination.

Namely, the present invention provides a recombinant DNA autonomously replicable in cells of coryneform bacte-

ria, comprising a DNA sequence coding for an aspartokinase in which feedback inhibition by L-lysine and L-threonine is substantially desensitized, a DNA sequence coding for a dihydrodipicolinate reductase, a DNA sequence coding for dihydrodipicolinate synthase, a DNA sequence coding for diaminopimelate decarboxylase, and a DNA sequence coding for aspartate aminotransferase.

In another aspect, the present invention provides a coryneform bacterium harboring an aspartokinase in which feedback inhibition by L-lysine and L-threonine is substantially desensitized, and comprising an enhanced DNA sequence coding for a dihydrodipicolinate reductase, an enhanced DNA sequence coding for dihydrodipicolinate reductase, an enhanced DNA sequence coding for dihydrodipicolinate synthase, an enhanced DNA sequence coding for diaminopimelate decarboxylase and an enhanced DNA sequence coding for aspartate aminotransferase.

In still another aspect, the present invention provides a method for producing L-lysine comprising the steps of cultivating any one of the coryneform bacteria described above in an appropriate medium, to allow L-lysine to be produced and accumulated in a culture of the bacterium, and collecting L-lysine from the culture.

The present invention also provides a DNA coding for a protein comprising an amino acid sequence shown in SEQ ID NO: 31. An example of the DNA is a DNA comprising a nucleotide sequence of nucleotide number 879 to 2174 in a nucleotide sequence shown in SEQ ID NO: 30.

The present invention further provides a vector pVK7, which is autonomously replicable in cells of *Escherichia coli* and *Brevibacterium lactofermentum*, and comprising a multiple cloning site and *lacZ'*.

The coryneform bacteria referred to in the present invention are a group of microorganisms as defined in *Bergey's Manual of Determinative Bacteriology*, 8th ed., p. 599 (1974), which are aerobic Gram-positive non-acid-fast rods having no spore-forming ability. The coryneform bacteria include bacteria belonging to the genus *Corynebacterium*, bacteria belonging to the genus *Brevibacterium* having been hitherto classified into the genus *Brevibacterium* but united as bacteria belonging to the genus *Corynebacterium* at present, and bacteria belonging to the genus *Brevibacterium* closely relative to bacteria belonging to the genus *Corynebacterium*.

According to the present invention, the L-lysine productivity of coryneform bacteria can be improved.

BRIEF EXPLANATION OF THE DRAWINGS

Fig. 1 illustrates a process of construction of plasmids p399AK9B and p399AKYB comprising mutant *lysC*.

Fig. 2 illustrates a process of construction of a plasmid pDPRB comprising *dapB* and Brevi.-ori.

Fig. 3 illustrates a process of construction of a plasmid pDPSB comprising *dapA* and Brevi.-ori.

Fig. 4 illustrates a process of construction of a plasmid p299LYSA comprising *lysA*.

Fig. 5 illustrates a process of construction of a plasmid pLYSAB comprising *lysA* and Brevi.-ori.

Fig. 6 illustrates a process of construction of a plasmid pCRCAB comprising *lysC*, *dapB* and Brevi.-ori.

Fig. 7 illustrates a process of construction of a plasmid pCB comprising mutant *lysC* and *dapB*.

Fig. 8 illustrates a process of construction of a plasmid pAB comprising *dapA*, *dapB* and Brevi.-ori.

Fig. 9 illustrates a process of construction of a plasmid pCAB comprising mutant *lysC*, *dapA*, *dapB*, and Brevi.-ori.

Fig. 10 illustrates a process of construction of a plasmid pCABL comprising mutant *lysC*, *dapA*, *dapB*, *lysA*, and Brevi.-ori.

Fig. 11 illustrates a process of construction of novel cloning vectors for Coryneform bacteria, pVK6 and pVK7.

Fig. 12 illustrates a process of construction of a plasmid pOm comprising *aspC*.

Fig. 13 illustrates two ORFs on an ATCC 13869 chromosomal DNA fragment.

Fig. 14 illustrates a process of construction of pORF1.

DETAILED DESCRIPTION OF THE INVENTION

(1) Preparation of genes for L-lysine biosynthesis used for the present invention

The genes for L-lysine biosynthesis used in the present invention are obtained respectively by preparing chromosomal DNA from a bacterium as a DNA donor, constructing a chromosomal DNA library by using a plasmid vector or the like, selecting a strain harboring a desired gene, and recovering, from the selected strain, recombinant DNA into which the gene has been inserted. The DNA donor for the gene for L-lysine biosynthesis used in the present invention is not specifically limited provided that the desired gene for L-lysine biosynthesis expresses an enzyme protein which functions in cells of coryneform bacteria. However, the DNA donor is preferably a coryneform bacterium.

All of the genes of *lysC*, *dapA*, *dapB* and *lysA* originating from coryneform bacteria have known sequences. Accordingly, they can be obtained by performing amplification in accordance with the polymerase chain reaction method (PCR; see White, T. J. et al., *Trends Genet.*, 5, 185 (1989)).

Each of the genes for L-lysine biosynthesis used in the present invention is obtainable in accordance with certain methods as exemplified below.

(1) Preparation of mutant *lysC*

A DNA fragment containing mutant *lysC* can be prepared from a mutant strain in which synergistic feedback inhibition on the AK activity by L-lysine and L-threonine is substantially desensitized (International Publication Pamphlet of WO 94/25605). Such a mutant strain can be obtained, for example, from a group of cells originating from a wild type strain of a coryneform bacterium subjected to a mutation treatment by applying an ordinary mutation treatment such as ultraviolet irradiation and treatment with a mutating agent such as N-methyl-N'-nitro-N-nitrosoguanidine (NTG). The AK activity can be measured by using a method described by Miyajima, R. et al. in *The Journal of Biochemistry* (1968), 63(2), 139-148. The most preferred as such a mutant strain is represented by an L-lysine-producing bacterium AJ3445 (FERM P-1944) derived by a mutation treatment from a wild type strain of *Brevibacterium lactofermentum* ATCC 13869 (having its changed present name of *Corynebacterium glutamicum*).

Alternatively, mutant *lysC* is also obtainable by an *in vitro* mutation treatment of plasmid DNA containing wild type *lysC*. In another aspect, information is specifically known on mutation to desensitize synergistic feedback inhibition on AK by L-lysine and L-threonine (International Publication Pamphlet of WO 94/25605). Accordingly, mutant *lysC* can be also prepared from wild type *lysC* on the basis of the information in accordance with, for example, the site-directed mutagenesis method.

A fragment comprising *lysC* can be isolated from a coryneform bacterium by preparing chromosomal DNA in accordance with, for example, a method of Saito and Miura (H. Saito and K. Miura, *Biochem. Biophys. Acta*, 72, 619 (1963)), and amplifying *lysC* in accordance with the polymerase chain reaction method (PCR; see White, T. J. et al., *Trends Genet.*, 5, 185 (1989)).

DNA primers are exemplified by single strand DNA's of 23-mer and 21-mer having nucleotide sequences shown in SEQ ID NOs: 1 and 2 in Sequence Listing in order to amplify, for example, a region of about 1,643 bp coding for *lysC* based on a sequence known for *Corynebacterium glutamicum* (see *Molecular Microbiology* (1991), 5(5), 1197-1204; *Mol. Gen. Genet.* (1990), 224, 317-324). DNA can be synthesized in accordance with an ordinary method by using DNA synthesizer model 380B produced by Applied Biosystems and using the phosphoramidite method (see *Tetrahedron Letters* (1981), 22, 1859). PCR can be performed by using DNA Thermal Cycler Model PJ2000 produced by Takara Shuzo, and using Taq DNA polymerase in accordance with a method designated by the supplier.

It is preferred that *lysC* amplified by PCR is ligated with vector DNA autonomously replicable in cells of *E. coli* and/or coryneform bacteria to prepare recombinant DNA, and the recombinant DNA is introduced into cells of *E. coli* beforehand. Such provision makes following operations easy. The vector autonomously replicable in cells of *E. coli* is preferably a plasmid vector which is preferably autonomously replicable in cells of a host, including, for example, pUC19, pUC18, pBR322, pHSG299, pHSG399, pHSG398, and RSF1010.

When a DNA fragment having an ability to allow a plasmid to be autonomously replicable in coryneform bacteria is inserted into these vectors, they can be used as a shuttle vector autonomously replicable in both *E. coli* and coryneform bacteria.

Such a shuttle vector includes the followings. Microorganisms harboring each of vectors and accession numbers in international deposition authorities (in parentheses) are shown.

pHC4: *Escherichia coli* AJ12617 (FERM BP-3532)
 pAJ655: *Escherichia coli* AJ11882 (FERM BP-136) *Corynebacterium glutamicum* SR8201 (ATCC 39135)
 pAJ1844: *Escherichia coli* AJ11883 (FERM BP-137) *Corynebacterium glutamicum* SR8202 (ATCC 39136)
 pAJ611: *Escherichia coli* AJ11884 (FERM BP-138)
 pAJ3148: *Corynebacterium glutamicum* SR8203 (ATCC 39137)
 pAJ440: *Bacillus subtilis* AJ11901 (FERM BP-140)

These vectors are obtainable from the deposited microorganisms as follows. Cells collected at a logarithmic growth phase were lysed by using lysozyme and SDS, followed by separation from a lysate by centrifugation at 30,000 × g to obtain a supernatant. To the supernatant, polyethylene glycol is added, followed by fractionation and purification by means of cesium chloride-ethidium bromide equilibrium density gradient centrifugation.

E. coli can be transformed by introducing a plasmid in accordance with, for example, a method of D. M. Morrison (*Methods in Enzymology*, 68, 326 (1979)) or a method in which recipient cells are treated with calcium chloride to increase permeability for DNA (Mandel, M. and Higa, A., *J. Mol. Biol.*, 53, 159 (1970)).

Wild type *lysC* is obtained when *lysC* is isolated from an AK wild type strain, while mutant *lysC* is obtained when *lysC* is isolated from an AK mutant strain in accordance with the method as described above.

An example of a nucleotide sequence of a DNA fragment containing wild type *lysC* is shown in SEQ ID NO: 3 in Sequence Listing. An amino acid sequence of α -subunit of a wild type AK protein is deduced from the nucleotide sequence, and is shown in SEQ ID NO: 4 in Sequence Listing together with the DNA sequence. Only the amino acid sequence is shown in SEQ ID NO: 5. An amino acid sequence of β -subunit of the wild type AK protein is deduced from

the nucleotide sequence of DNA, and is shown in SEQ ID NO: 6 in Sequence Listing together with the DNA sequence. Only the amino acid sequence is shown in SEQ ID NO: 7. In each of the subunits, GTG is used as an initiation codon, and a corresponding amino acid is represented by methionine. However, this representation refers to methionine, valine, or formylmethionine.

5 The mutant *lysC* used in the present invention is not specifically limited provided that it codes for AK in which synergistic feedback inhibition by L-lysine and L-threonine is desensitized. However, the mutant *lysC* is exemplified by one including mutation in which an amino acid residue corresponding to a 279th alanine residue as counted from the N-terminal is changed into an amino acid residue other than alanine and other than acidic amino acid in the α -subunit, and an amino acid residue corresponding to a 30th alanine residue from the N-terminal is changed into an amino acid residue other than alanine and other than acidic amino acid in the β -subunit in the amino acid sequence of the wild type AK. The amino acid sequence of the wild type AK specifically includes the amino acid sequence shown in SEQ ID NO: 5 in Sequence Listing as the α -subunit, and the amino acid sequence shown in SEQ ID NO: 7 in Sequence Listing as the β -subunit.

Those preferred as the amino acid residue other than alanine and other than acidic amino acid include threonine, arginine, cysteine, phenylalanine, proline, serine, tyrosine, and valine residues.

15 The codon corresponding to an amino acid residue to be substituted is not specifically limited for its type provided that it codes for the amino acid residue. It is predicted that the amino acid sequence of wild type AK may slightly differ depending on the difference in bacterial species and bacterial strains. AK's, which have mutation based on, for example, substitution, deletion, or insertion of one or more amino acid residues at one or more positions irrelevant to the enzyme activity as described above, can be also used for the present invention. A DNA coding for AK having the spontaneous mutation can be obtained by isolating a DNA which is hybridizable with, for example, the DNA having a part of the nucleotide sequence shown in SEQ ID NO: 3 under the stringent condition. By the "stringent condition" referred to herein is meant a condition under which a specific hybrid is formed, and nonspecific hybrid is not formed. It is difficult to clearly express the condition with numerical values. However, the condition is exemplified by a condition under which, 25 nucleic acid having high homology, for example, DNA's having homology of not less than 90% are hybridized with each other, and nucleic acids having homology lower than the above are not hybridized with each other, or a condition of a temperature of from a melting out temperature (T_m) of a completely-matched hybrid to $(T_m - 30)^\circ\text{C}$, preferably from $T_m - 10$ to $(T_m - 20)^\circ\text{C}$ and a salt concentration corresponding to $1 \times \text{SSC}$, preferably $0.1 \times \text{SSC}$.

Other AK's, which have artificial mutation based on, for example, substitution, deletion, or insertion of other one or more amino acid residues, can be also used provided that no influence is substantially exerted on the AK activity, and on the desensitization of synergistic feedback inhibition by L-lysine and L-threonine. A DNA coding for AK having the artificial mutation can be obtained by modifying the nucleotide sequence to give substitution, deletion or insertion of a specified site by, for example, site-specific mutagenesis. Also, *lysC* having the mutation can be obtained by known mutagen treatment. The mutagen treatment includes in vitro treatment of a DNA containing *lysC* with hydroxylamine or the like, and treatment of microorganism harboring a DNA containing *lysC* with a mutagen such as ultraviolet irradiation or a mutagenic agent used for ordinary artificial mutagenesis such as N-methyl-N'-nitro-N-nitrosoguanidine (NTG) or nitric acid. After the mutagen treatment, a site to which mutation is introduced or in which mutation occurs can be determined by selecting a DNA or a microorganism which codes for or produces AK which has the AK activity and whose amino acid sequence is mutated from the DNA subjected to the mutagen treatment or the microorganism subjected to the mutagen treatment. A site of the introduced mutation is not specifically restricted provided that no influence is substantially exerted on the AK activity and on desensitization of feedback inhibition. A number of the introduced mutation varies depending on a site or a kind of the mutated amino acid in a steric structure of a protein, and is not specifically restricted provided that no influence is substantially exerted on the AK activity and on desensitization of feedback inhibition. The number is usually 1 to 20, preferably 1 to 10.

45 An amino acid residue corresponding to the specified alanine residue in the amino acid sequence of AK having the mutation as described above can be easily determined by one skilled in the art.

An AJ12691 strain obtained by introducing a mutant *lysC* plasmid p399AK9B into an AJ12036 strain (FERM BP-734) as a wild type strain of *Brevibacterium lactofermentum* has been deposited on April 10, 1992 under an accession number of FERM P-12918 in National Institute of Bioscience and Human Technology of Agency of Industrial Science and Technology of Ministry of International Trade and Industry (1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, 305 Japan), transferred to international deposition based on the Budapest Treaty on February 10, 1995, and deposited under an accession number of FERM BP-4999.

(2) Preparation of *dapB*

55 A DNA fragment containing *dapB* can be prepared from chromosome of a coryneform bacterium by means of PCR. The DNA donor is not specifically limited, however, it is exemplified by *Brevibacterium lactofermentum* ATCC 13869 strain.

A DNA sequence coding for DDPR is known for Brevibacterium lactofermentum (Journal of Bacteriology, 175(9), 2743-2749 (1993)), on the basis of which DNA primers for PCR can be prepared. Such DNA primers are specifically exemplified by DNA's of 23-mers respectively having nucleotide sequences depicted in SEQ ID NOs: 8 and 9 in Sequence Listing. Synthesis of DNA, PCR, and preparation of a plasmid containing obtained dapB can be performed in the same manner as those for lysC described above.

A nucleotide sequence of a DNA fragment containing dapB and an amino acid sequence deduced from the nucleotide sequence are illustrated in SEQ ID NO: 10. Only the amino acid sequence is shown in SEQ ID NO: 11. In addition to DNA fragments coding for this amino acid sequence, the present invention can equivalently use DNA fragments coding for amino acid sequences substantially the same as the amino acid sequence shown in SEQ ID NO: 11, namely amino acid sequences having mutation based on, for example, substitution, deletion, or insertion of one or more amino acids provided that there is no substantial influence on the DDPR activity. The dapB having spontaneous or artificial mutation can be obtained in the same manner as those for the DNA coding for AK having mutation which exerts no influence on the AK activity and on the desensitization of synergistic feedback inhibition by L-lysine and L-threonine.

A transformant strain AJ13107 obtained by introducing a plasmid pCRDAPB containing dapB obtained in Example described later on into E. coli JM109 strain has been internationally deposited since May 26, 1995 under an accession number of FERM BP-5114 in National Institute of Bioscience and Human Technology of Agency of Industrial Science and Technology of Ministry of International Trade and Industry (1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, 305 Japan) based on the Budapest Treaty.

(3) Preparation of dapA

A DNA fragment containing dapA can be prepared from chromosome of a coryneform bacterium by means of PCR. The DNA donor is not specifically limited, however, it is exemplified by Brevibacterium lactofermentum ATCC 13869 strain.

A DNA sequence coding for DDPS is known for Corynebacterium glutamicum (see Nucleic Acids Research, 18(21), 6421 (1990); EMBL accession No. X53993), on the basis of which DNA primers for PCR can be prepared. Such DNA primers are specifically exemplified by DNA's of 23-mers respectively having nucleotide sequences depicted in SEQ ID NOs: 12 and 13 in Sequence Listing. Synthesis of DNA, PCR, and preparation of a plasmid containing obtained dapA can be performed in the same manner as those for lysC described above.

A nucleotide sequence of a DNA fragment containing dapA and an amino acid sequence deduced from the nucleotide sequence are exemplified in SEQ ID NO: 14. Only the amino acid sequence is shown in SEQ ID NO: 15. In addition to DNA fragments coding for this amino acid sequence, the present invention can equivalently use DNA fragments coding for amino acid sequences substantially the same as the amino acid sequence shown in SEQ ID NO: 15, namely amino acid sequences having mutation based on, for example, substitution, deletion, or insertion of one or more amino acids provided that there is no substantial influence on the DDPS activity. The dapA having spontaneous or artificial mutation can be obtained in the same manner as those for the DNA coding for AK having mutation which exerts no influence on the AK activity and on the desensitization of synergistic feedback inhibition by L-lysine and L-threonine.

A transformant strain AJ13106 obtained by introducing a plasmid pCRDAPA containing dapA obtained in Example described later on into E. coli JM109 strain has been internationally deposited since May 26, 1995 under an accession number of FERM BP-5113 in National Institute of Bioscience and Human Technology of Agency of Industrial Science and Technology of Ministry of International Trade and Industry (1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, 305 Japan) based on the Budapest Treaty.

(14) Preparation of lysA

A DNA fragment containing lysA can be prepared from chromosome of a coryneform bacterium by means of PCR. The DNA donor is not specifically limited, however, it is exemplified by Brevibacterium lactofermentum ATCC 13869 strain.

In the coryneform bacteria, lysA forms an operon together with argS (arginyl-tRNA synthase gene), and lysA exists downstream from argS. Expression of lysA is regulated by a promoter existing upstream from argS (see Journal of Bacteriology, Nov., 7356-7362 (1993)). DNA sequences of these genes are known for Corynebacterium glutamicum (see Molecular Microbiology, 4(11), 1819-1830 (1990); Molecular and General Genetics, 212, 112-119 (1988)), on the basis of which DNA primers for PCR can be prepared. Such DNA primers are specifically exemplified by DNA's of 23-mers respectively having nucleotide sequences shown in SEQ ID NO: 16 in Sequence Listing (corresponding to nucleotide numbers 11 to 33 in a nucleotide sequence described in Molecular Microbiology, 4(11), 1819-1830 (1990)) and SEQ ID NO: 17 (corresponding to nucleotide numbers 1370 to 1392 in a nucleotide sequence described in Molecular and General Genetics, 212, 112-119 (1988)). Synthesis of DNA, PCR, and preparation of a plasmid containing obtained lysA can be performed in the same manner as those for lysC described above.

In Example described later on, a DNA fragment containing a promoter, argS, and lysA was used in order to enhance lysA. However, argS is not essential for the present invention. It is allowable to use a DNA fragment in which lysA is ligated just downstream from a promoter.

A nucleotide sequence of a DNA fragment containing argS and lysA, and an amino acid sequence deduced to be encoded by the nucleotide sequence are exemplified in SEQ ID NO: 18. An example of an amino acid sequence encoded by argS is shown in SEQ ID NO: 19, and an example of an amino acid sequence encoded by lysA is shown in SEQ ID NO: 20. In addition to DNA fragments coding for these amino acid sequences, the present invention can equivalently use DNA fragments coding for amino acid sequences substantially the same as the amino acid sequence shown in SEQ ID NO: 20, namely amino acid sequences having mutation based on, for example, substitution, deletion, or insertion of one or more amino acids provided that there is no substantial influence on the DDC activity. The lysA having spontaneous or artificial mutation can be obtained in the same manner as those for the DNA coding for AK having mutation which exerts no influence on the AK activity and on the desensitization of synergistic feedback inhibition by L-lysine and L-threonine.

(5) Preparation of aspC

A DNA fragment containing aspC can be prepared from a gene library prepared from chromosome of a microorganism such as a coryneform bacterium and a bacterium belonging to the genus Escherichia by using complementarity to an auxotrophic property of an AAT-deficient strain as an indication. The DNA donor of the coryneform bacterium is not specifically limited, however, it is exemplified by Brevibacterium lactofermentum ATCC 13869 strain. The DNA donor of the bacterium belonging to the genus Escherichia is not specifically limited, however, it is exemplified by E. coli JM109 strain.

Specifically, a method for preparing aspC of coryneform bacteria is known (Japanese Patent Publication No. 6-102028) and aspC can be prepared according to this method.

A DNA sequence coding for AAT is known for E. coli (Kuramitsu, S. et al., J. Biochem., 97(4), 1259-1262 (1985)), on the basis of which primers for PCR can be prepared. Such DNA primers are specifically exemplified by DNA's of 20-mers respectively having nucleotide sequences depicted in SEQ ID NOs: 21 and 22 in Sequence Listing. Synthesis of DNA, PCR, and preparation of a plasmid containing obtained aspC can be performed in the same manner as those for lysC described above.

A nucleotide sequence of a DNA fragment containing aspC and an amino acid sequence deduced from the nucleotide sequence are illustrated in SEQ ID NO: 23. Only the amino acid sequence is shown in SEQ ID NO: 24. Another nucleotide sequence of a DNA fragment containing aspC and an amino acid sequence deduced from the nucleotide sequence are illustrated in SEQ ID NO: 30. Only the amino acid sequence is shown in SEQ ID NO: 31. In addition to DNA fragments coding for this amino acid sequence, the present invention can equivalently use DNA fragments coding for amino acid sequences substantially the same as the amino acid sequence shown in SEQ ID NO: 24 or 31, namely amino acid sequences having mutation based on, for example, substitution, deletion, or insertion of one or more amino acids provided that there is no substantial influence on the AAT activity. The aspC having spontaneous or artificial mutation can be obtained in the same manner as those for the DNA coding for AK having mutation which exerts no influence on the AK activity and on the desensitization of synergistic feedback inhibition by L-lysine and L-threonine.

The aspC having the nucleotide sequence shown in SEQ ID NO: 30 originates from Corynebacterium lactofermentum, and has been firstly obtained according to the method described in Example 9 described below by the present invention. Thus, the present invention provides a DNA coding for a protein comprising the amino acid sequence shown in SEQ ID NO: 31. An example of the DNA includes a DNA comprising a nucleotide sequence of nucleotide number 879 to 2174 in a nucleotide sequence shown in SEQ ID NO: 30.

(2) Recombinant DNA and coryneform bacterium of the present invention

The coryneform bacterium of the present invention harbors an aspartokinase (mutant AK) in which feedback inhibition by L-lysine and L-threonine is substantially desensitized, wherein the DNA sequence coding for a dihydrodipicolinate reductase, the DNA sequence coding for a dihydrodipicolinate synthase, the DNA sequence coding for a diaminopimelate decarboxylase and the DNA coding for an aspartate aminotransferase are enhanced.

The term "enhance" herein refers to the fact that the intracellular activity of an enzyme encoded by the DNA is raised by, for example, increasing the copy number of a gene, using a strong promoter, using a gene coding for an enzyme having a high specific activity, or combining these means.

The coryneform bacterium harboring the mutant AK may be those which produce the mutant aspartokinase as a result of mutation, or those which are transformed by introducing mutant lysC.

Examples of the coryneform bacterium used to introduce the DNA described above include, for example, the following lysine-producing wild type strains:

Corynebacterium acetoacidophilum ATCC 13870;
Corynebacterium acetoglutamicum ATCC 15806;
Corynebacterium callunae ATCC 15991;
Corynebacterium glutamicum ATCC 13032;
5 (Brevibacterium divaricatum) ATCC 14020;
(Brevibacterium lactofermentum) ATCC 13869;
(Corynebacterium lilium) ATCC 15990;
(Brevibacterium flavum) ATCC 14067;
Corynebacterium melassecola ATCC 17965;
10 Brevibacterium saccharolyticum ATCC 14066;
Brevibacterium immariophilum ATCC 14068;
Brevibacterium roseum ATCC 13825;
Brevibacterium thiogenitalis ATCC 19240;
Microbacterium ammoniaphilum ATCC 15354;
15 Corynebacterium thermoaminogenes AJ12340 (FERM BP-1539);

Other than the bacterial strains described above, those usable as a host include, for example, mutant strains having an L-lysine-producing ability derived from the aforementioned strains. Such artificial mutant strains includes the follow-
ings: S-(2-aminoethyl)cysteine (hereinafter abbreviated as "AEC") resistant mutant strains (for example, Brevibacterium
20 lactofermentum AJ11082 (NRRL B-1147), Japanese Patent Publication Nos. 56-1914, 56-1915, 57-14157, 57-14158,
57-30474, 58-10075, 59-4993, 61-35840, 62-24074, 62-36673, 5-11958, 7-112437, and 7-112438); mutant strains
which require amino acid such as L-homoserine for their growth (Japanese Patent Publication Nos. 48-28078 and 56-
6499); mutant strains which exhibit resistance to AEC and require amino acids such as L-leucine, L-homoserine, L-pro-
line, L-serine, L-arginine, L-alanine, and L-valine (United States Patent Nos. 3,708,395 and 3,825,472); L-lysine-pro-
25 ducing mutant strains which exhibit resistance to DL- α -amino- ϵ -caprolactam, α -amino-lauryllactam, aspartate-analog,
sulfa drug, quinoid, and N-lauroylleucine; L-lysine-producing mutant strains which exhibit resistance to inhibitors of oxy-
aloacetate decarboxylase or respiratory system enzymes (Japanese Patent Application Laid-open Nos. 50-53588, 50-
31093, 52-102498, 53-9394, 53-86089, 55-9783, 55-9759, 56-32995 and 56-39778, and Japanese Patent Publication
Nos. 53-43591 and 53-1833); L-lysine-producing mutant strains which require inositol or acetic acid (Japanese Patent
30 Application Laid-open Nos. 55-9784 and 56-8692); L-lysine-producing mutant strains which exhibit sensitivity to fluoro-
pyruvic acid or temperature not less than 34°C (Japanese Patent Application Laid-open Nos. 55-9783 and 53-86090);
and producing mutant strains belonging to the genus Brevibacterium or Corynebacterium which exhibit resistance to
ethylene glycol and produce L-lysine (United States Patent No. 4,411,997).

In a specified embodiment, in order to enhance the genes for L-lysine biosynthesis in the host as described above,
35 the genes are introduced into the host by using a plasmid vector, transposon or phage vector or the like. Upon the intro-
duction, it is expected to make enhancement to some extent even by using a low copy type vector. However, it is pre-
ferred to use a multiple copy type vector. Such a vector includes, for example, plasmid vectors, pAJ655, pAJ1844,
pAJ611 pAJ3148, and pAJ440 described above. Besides, transposons derived from coryneform bacteria are described
in International Publication Pamphlets of WO02/02627 and WO93/18151, European Patent Publication No. 445385,
40 Japanese Patent Application Laid-open No. 6-46867, Vertes, A. A. et al., Mol. Microbiol., 11, 739-746 (1994), Bonamy,
C., et al., Mol. Microbiol., 14, 571-581 (1994), Vertes, A. A. et al., Mol. Gen. Genet., 245, 397-405 (1994), Jagar, W. et
al., FEMS Microbiology Letters, 126, 1-6 (1995), Japanese Patent Application Laid-open No. 7-107976, Japanese Pat-
ent Application Laid-open No. 7-327680 and the like.

In the present invention, it is not indispensable that the mutant lysC is necessarily enhanced. It is allowable to use
45 those which have mutation on lysC on chromosomal DNA, or in which the mutant lysC is incorporated into chromo-
somal DNA. Alternatively, the mutant lysC may be introduced by using a plasmid vector. On the other hand, dapA,
dapB, lysA, and aspC are preferably enhanced in order to efficiently produce L-lysine.

Each of the genes of lysC, dapA, dapB, lysA, and aspC may be successively introduced into the host by using dif-
ferent vectors respectively. Alternatively, two, three, four, or five species of the genes may be introduced together by
50 using a single vector. When different vectors are used, the genes may be introduced in any order, however, it is pre-
ferred to use vectors which have a stable sharing and harboring mechanism in the host, and which are capable of co-
existing with each other.

Particularly, as a vector for introducing aspC into coryneform bacteria, a vector pVK7 is preferably used. The vector
pVK7 is a cloning vector for coryneform bacteria provided by the present invention, which is autonomously replicable in
55 cells of Escherichia coli and Brevibacterium lactofermentum, and comprising a multiple cloning site and lacZ'. The vec-
tor pVK7 can be constructed according to the method described in Example 8 described below.

A coryneform bacterium harboring the mutant AK and further comprising enhanced dapB, dapA, lysA and aspC is
obtained, for example, by introducing, into a host coryneform bacterium, a recombinant DNA containing mutant lysC

and dapB, dapA, lysA and aspC autonomously replicable in cells of coryneform bacteria.

The above-mentioned recombinant DNAs can be obtained, for example, by inserting each of the genes participating in L-lysine biosynthesis into a vector such as plasmid vector, transposon or phage vector as described above.

In the case in which a plasmid is used as a vector, the recombinant DNA can be introduced into the host in accordance with an electric pulse method (Sugimoto et al., Japanese Patent Application Laid-open No. 2-207791). Amplification of a gene using transposon can be performed by introducing a plasmid which carrying a transposon into the host cell and inducing transposition of the transposon.

In coryneform bacteria used in the present invention, a gene participating in L-lysine biosynthesis such as a DNA sequence coding for a phosphoenolpyruvate carboxylase and a DNA sequence coding for a diaminopimelate dehydrogenase may be enhanced in addition to the above-mentioned genes.

(3) Method for producing L-lysine

L-Lysine can be efficiently produced by cultivating, in an appropriate medium, the coryneform bacterium comprising the enhanced genes for L-lysine biosynthesis as described above, to allow L-lysine to be produced and accumulated in a culture of the bacterium, and collecting L-lysine from the culture.

The medium to be used is exemplified by an ordinary medium containing a carbon source, a nitrogen source, inorganic ions, and optionally other organic components.

As the carbon source, it is possible to use sugars such as glucose, fructose, sucrose, molasses, and starch hydrolysate; and organic acids such as fumaric acid, citric acid, and succinic acid.

As the nitrogen source, it is possible to use inorganic ammonium salts such as ammonium sulfate, ammonium chloride, and ammonium phosphate; organic nitrogen such as soybean hydrolysate; ammonia gas; and aqueous ammonia.

As organic trace nutrient sources, it is desirable to contain required substances such as vitamin B₁ and L-homoserine or yeast extract or the like in appropriate amounts. Other than the above, potassium phosphate, magnesium sulfate, iron ion, manganese ion and so on are added in small amounts, if necessary.

Cultivation is preferably carried out under an aerobic condition for about 30 to 90 hours. The cultivation temperature is preferably controlled at 25°C to 37°C, and pH is preferably controlled at 5 to 8 during cultivation. Inorganic or organic, acidic or alkaline substances, or ammonia gas or the like can be used for pH adjustment. L-lysine can be collected from a culture by combining an ordinary ion exchange resin method, a precipitation method, and other known methods.

EXAMPLES

The present invention will be more specifically explained below with reference to Examples.

Example 1: Preparation of Wild Type lysC Gene and Mutant lysC Gene from Brevibacterium lactofermentum

(1) Preparation of wild type and mutant lysC's and preparation of plasmids containing them

A strain of Brevibacterium lactofermentum ATCC 13869, and an L-lysine-producing mutant strain AJ3445 (FERM P-1944) obtained from the ATCC 13869 strain by a mutation treatment were used as chromosomal DNA donors. The AJ3445 strain had been subjected to mutation so that lysC was changed to involve substantial desensitization from concerted inhibition by lysine and threonine (Journal of Biochemistry, **68**, 701-710 (1970)).

A DNA fragment containing lysC was amplified from chromosomal DNA in accordance with the PCR method (polymerase chain reaction; see White, T. J. et al., Trends Genet., **5**, 185 (1989)). As for DNA primers used for amplification, single strand DNA's of 23-mer and 21-mer having nucleotide sequences shown in SEQ ID NOs: 1 and 2 were synthesized in order to amplify a region of about 1,643 bp coding for lysC on the basis of a sequence known for Corynebacterium glutamicum (see Molecular Microbiology (1991), **5**(5), 1197-1204; and Mol. Gen. Genet. (1990), **224**, 317-324). DNA was synthesized in accordance with an ordinary method by using DNA synthesizer model 380B produced by Applied Biosystems and using the phosphoramidite method (see Tetrahedron Letters (1981), **22**, 1859).

The gene was amplified by PCR by using DNA Thermal Cycler Model PJ2000 produced by Takara Shuzo, and using Taq DNA polymerase in accordance with a method designated by the supplier. An amplified gene fragment of 1,643 kb was confirmed by agarose gel electrophoresis. After that, the fragment excised from the gel was purified in accordance with an ordinary method, and it was digested with restriction enzymes NruI (produced by Takara Shuzo) and EcoRI (produced by Takara Shuzo).

pHSG399 (see Takeshita, S. et al., Gene (1987), **61**, 63-74) was used as a cloning vector for the gene fragment. pHSG399 was digested with restriction enzymes SmaI (produced by Takara Shuzo) and EcoRI, and it was ligated with the amplified lysC fragment. DNA was ligated by using DNA ligation kit (produced by Takara Shuzo) in accordance with a designated method. Thus plasmids were prepared, in which the lysC fragments amplified from chromosomes of

Brevibacterium lactofermentum were ligated with pHSG399 respectively. A plasmid comprising *lysC* from ATCC 13869 (wild type strain) was designated as p399AKY, and a plasmid comprising *lysC* from AJ3463 (L-lysine-producing bacterium) was designated as p399AK9.

A DNA fragment (hereinafter referred to as "Brevi.-ori") having an ability to make a plasmid autonomously replicable in bacteria belonging to the genus *Corynebacterium* was introduced into p399AKY and p399AK9 respectively to prepare plasmids carrying *lysC* autonomously replicable in bacteria belonging to the genus *Corynebacterium*. Brevi.-ori was prepared from a plasmid vector pHK4 containing Brevi.-ori and autonomously replicable in cells of both *Escherichia coli* and bacteria belonging to the genus *Corynebacterium*. pHK4 was constructed by digesting pHK4 with *Kpn*I (produced by Takara Shuzo) and *Bam*HI, (produced by Takara Shuzo), extracting a Brevi.-ori fragment, and ligating it with pHSG298 having been also digested with *Kpn*I and *Bam*HI (see Japanese Patent Application Laid-open No. 5-7491). pHK4 gives kanamycin resistance to a host. *Escherichia coli* harboring pHK4 was designated as *Escherichia coli* AJ13136, and deposited on August 1, 1995 under an accession number of FERM BP-5186 in National Institute of Bioscience and Human Technology of Agency of Industrial Science and Technology of Ministry of International Trade and Industry (1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, 305 Japan).

pHK4 was digested with restriction enzymes *Kpn*I and *Bam*HI, and cleaved edges were blunt-ended. Blunt end formation was performed by using DNA Blunting kit (produced by Takara Shuzo) in accordance with a designated method. After the blunt end formation, a phosphorylated *Bam*HI linker (produced by Takara Shuzo) was ligated to make modification so that the DNA fragment corresponding to the Brevi.-ori portion might be excised from pHK4 by digestion with only *Bam*HI. This plasmid was digested with *Bam*HI, and the generated Brevi.-ori DNA fragment was ligated with p399AKY and p399AK9 having been also digested with *Bam*HI respectively to prepare plasmids each containing the *lysC* gene autonomously replicable in bacteria belonging to the genus *Corynebacterium*.

A plasmid containing the wild type *lysC* gene originating from p399AKY was designated as p399AKYB, and a plasmid containing the mutant *lysC* gene originating from p399AK9 was designated as p399AK9B. The process of construction of p399AK9B and p399AKYB is shown in Fig. 1. A strain AJ12691 obtained by introducing the mutant *lysC* plasmid p399AK9B into a wild type strain of *Brevibacterium lactofermentum* (AJ12036 strain, FERM BP-734) was deposited on April 10, 1992 under an accession number of FERM P-12918 in National Institute of Bioscience and Human Technology of Agency of Industrial Science and Technology of Ministry of International Trade and Industry (1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, 305 Japan), transferred to international deposition based on the Budapest Treaty on February 10, 1995, and deposited under an accession number of FERM BP-4999.

(2) Determination of nucleotide sequences of wild type *lysC* and mutant *lysC* from *Brevibacterium lactofermentum*

The plasmid p399AKY containing the wild type *lysC* and the plasmid p399AK9 containing the mutant *lysC* were prepared from the respective transformants to determine nucleotide sequences of the wild type and mutant *lysC*'s. Nucleotide sequence determination was performed in accordance with a method of Sanger et al. (for example, F. Sanger et al., *Proc. Natl. Acad. Sci.*, 74, 5463 (1977)).

The nucleotide sequence of wild type *lysC* encoded by p399AKY is shown in SEQ ID NO: 3 in Sequence Listing. On the other hand, the nucleotide sequence of mutant *lysC* encoded by p399AK9 had only mutation of one nucleotide such that 1051st G was changed into A in SEQ ID NO: 3 as compared with wild type *lysC*. It is known that *lysC* of *Corynebacterium glutamicum* has two subunits (α , β) encoded in an identical reading frame on an identical DNA strand (see Kalinowski, J. et al., *Molecular Microbiology* (1991) 5(5), 1197-1204). Judging from homology, it is assumed that the gene sequenced herein also has two subunits (α , β) encoded in an identical reading frame on an identical DNA strand.

An amino acid sequence of the α -subunit of the wild type AK protein deduced from the nucleotide sequence of DNA is shown in SEQ ID NO: 4 together with the DNA sequence. Only the amino acid sequence is shown in SEQ ID NO: 5. An amino acid sequence of the β -subunit of the wild type AK protein deduced from the nucleotide sequence of DNA is shown in SEQ ID NO: 6 together with the DNA sequence. Only the amino acid sequence is shown in SEQ ID NO: 7. In each of the subunits, GTG is used as an initiation codon, and a corresponding amino acid is represented by methionine. However, this representation refers to methionine, valine, or formylmethionine.

On the other hand, mutation on the sequence of mutant *lysC* means occurrence of amino acid residue substitution such that a 279th alanine residue of the α -subunit is changed into a threonine residue, and a 30th alanine residue of the β -subunit is changed into a threonine residue in the amino acid sequence of the wild type AK protein (SEQ ID NOs: 5, 7).

Example 2: Preparation of dapB from Brevibacterium lactofermentum(1) Preparation of dapB and construction of plasmid containing dapB

A wild type strain of Brevibacterium lactofermentum ATCC 13869 was used as a chromosomal DNA donor. Chromosomal DNA was prepared from the ATCC 13869 strain in accordance with an ordinary method. A DNA fragment containing dapB was amplified from the chromosomal DNA in accordance with PCR. As for DNA primers used for amplification, DNAs of 23-mers having nucleotide sequences shown in SEQ ID NOs: 8 and 9 in Sequence Listing respectively were synthesized in order to amplify a region of about 2.0 kb coding for DDPR on the basis of a sequence known for Brevibacterium lactofermentum (see Journal of Bacteriology, 175(9), 2743-2749 (1993)). Synthesis of DNA and PCR were performed in the same manner as described in Example 1. pCR-Script (produced by Invitrogen) was used as a cloning vector for the amplified gene fragment of 2,001 bp, and was ligated with the amplified dapB fragment. Thus a plasmid was constructed, in which the dapB fragment of 2,001 bp amplified from chromosome of Brevibacterium lactofermentum was ligated with pCR-Script. The plasmid obtained as described above, which had dapB originating from ATCC 13869, was designated as pCRDAPB. A transformant strain AJ13107 obtained by introducing pCRDAPB into E. coli JM109 strain has been internationally deposited since May 26, 1995 under an accession number of FERM BP-5114 in National Institute of Bioscience and Human Technology of Agency of Industrial Science and Technology of Ministry of International Trade and Industry (1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, 305 Japan) based on the Budapest Treaty.

A fragment of 1,101 bp containing a structural gene of DDPR was extracted by digesting pCRDAPB with EcoRV and SphI. This fragment was ligated with pHSG399 having been digested with HincII and SphI to prepare a plasmid. The prepared plasmid was designated as p399DPR.

Brevi.-ori was introduced into the prepared p399DPR to construct a plasmid carrying dapB autonomously replicable in coryneform bacteria. pHK4 was digested with a restriction enzyme KpnI (produced by Takara Shuzo), and cleaved edges were blunt-ended. Blunt end formation was performed by using DNA Blunting kit (produced by Takara Shuzo) in accordance with a designated method. After the blunt end formation, a phosphorylated BamHI linker (produced by Takara Shuzo) was ligated to make modification so that the DNA fragment corresponding to the Brevi.-ori portion might be excised from pHK4 by digestion with only BamHI. This plasmid was digested with BamHI, and the generated Brevi.-ori DNA fragment was ligated with p399DPR having been also digested with BamHI to prepare a plasmid containing dapB autonomously replicable in coryneform bacteria. The prepared plasmid was designated as pDPRB. The process of construction of pDPRB is shown in Fig. 2.

(2) Determination of nucleotide sequence of dapB from Brevibacterium lactofermentum

Plasmid DNA was prepared from the AJ13107 strain harboring p399DPR, and its nucleotide sequence was determined in the same manner as described in Example 1. A determined nucleotide sequence and an amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 10. Only the amino acid sequence is shown in SEQ ID NO: 11.

Example 3: Preparation of dapA from Brevibacterium lactofermentum(1) Preparation of dapA and construction of plasmid containing dapA

A wild type strain of Brevibacterium lactofermentum ATCC 13869 was used as a chromosomal DNA donor. Chromosomal DNA was prepared from the ATCC 13869 strain in accordance with an ordinary method. A DNA fragment containing dapA was amplified from the chromosomal DNA in accordance with PCR. As for DNA primers used for amplification, DNAs of 23-mers having nucleotide sequences shown in SEQ ID NOs: 12 and 13 in Sequence Listing respectively were synthesized in order to amplify a region of about 1.5 kb coding for DDPS on the basis of a sequence known for Corynebacterium glutamicum (see Nucleic Acids Research, 18(21), 6421 (1990); EMBL accession No. X53993). Synthesis of DNA and PCR were performed in the same manner as described in Example 1. pCR1000 (produced by Invitrogen, see Bio/Technology, 9, 657-663 (1991)) was used as a cloning vector for the amplified gene fragment of 1,411 bp, and was ligated with the amplified dapA fragment. Ligation of DNA was performed by using DNA ligation kit (produced by Takara Shuzo) in accordance with a designated method. Thus a plasmid was constructed, in which the dapA fragment of 1,411 bp amplified from chromosome of Brevibacterium lactofermentum was ligated with pCR1000. The plasmid obtained as described above, which had dapA originating from ATCC 13869, was designated as pCRDAPA.

A transformant strain AJ13106 obtained by introducing pCRDAPA into E. coli JM109 strain has been internationally deposited since May 26, 1995 under an accession number of FERM BP-5113 in National Institute of Bioscience and

Human Technology of Agency of Industrial Science and Technology of Ministry of International Trade and Industry (1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, 305 Japan) based on the Budapest Treaty.

Brevi.-ori was introduced into the prepared pCRDAPA to construct a plasmid carrying dapA autonomously replicable in coryneform bacteria. pHK4 was digested with restriction enzymes KpnI and BamHI (produced by Takara Shuzo), and cleaved edges were blunt-ended. Blunt end formation was performed by using DNA Blunting kit (produced by Takara Shuzo) in accordance with a designated method. After the blunt end formation, a phosphorylated SmaI linker (produced by Takara Shuzo) was ligated to make modification so that the DNA fragment corresponding to the Brevi.-ori portion might be excised from pHK4 by digestion with only SmaI. This plasmid was digested with SmaI, and the generated Brevi.-ori DNA fragment was ligated with pCRDAPA having been also digested with SmaI to prepare a plasmid containing dapA autonomously replicable in coryneform bacteria. This plasmid was designated as pDPSB. The process of construction of pDPSB(Km^r) is shown in Fig. 3.

(2) Determination of nucleotide sequence of dapA from Brevibacterium lactofermentum

Plasmid DNA was prepared from the AJ13106 strain harboring pCRDAPA, and its nucleotide sequence was determined in the same manner as described in Example 1. A determined nucleotide sequence and an amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 14. Only the amino acid sequence is shown in SEQ ID NO: 15.

Example 4: Preparation of lysA from Brevibacterium lactofermentum

(1) Preparation of lysA and construction of plasmid containing lysA

A wild type strain of Brevibacterium lactofermentum ATCC 13869 was used as a chromosomal DNA donor. Chromosomal DNA was prepared from the ATCC 13869 strain in accordance with an ordinary method. A DNA fragment containing argS, lysA, and a promoter of an operon containing them was amplified from the chromosomal DNA in accordance with PCR. As for DNA primers used for amplification, synthetic DNAs of 23-mers having nucleotide sequences shown in SEQ ID NOs: 16 and 17 in Sequence Listing respectively were used in order to amplify a region of about 3.6 kb coding for arginyl-tRNA synthase and DDC on the basis of a sequence known for Corynebacterium glutamicum (see Molecular Microbiology, 4(11), 1819-1830 (1990); Molecular and General Genetics, 212, 112-119 (1988)). Synthesis of DNA and PCR were performed in the same manner as described in Example 1. pHSG399 was used as a cloning vector for the amplified gene fragment of 3,579 bp. pHSG399 was digested with a restriction enzyme SmaI (produced by Takara Shuzo), which was ligated with the DNA fragment containing amplified lysA. A plasmid obtained as described above, which had lysA originating from ATCC 13869, was designated as p399LYSA.

A DNA fragment containing lysA was extracted by digesting p399LYSA with KpnI (produced by Takara Shuzo) and BamHI (produced by Takara Shuzo). This DNA fragment was ligated with pHSG299 having been digested with KpnI and BamHI. An obtained plasmid was designated as p299LYSA. The process of construction of p299LYSA is shown in Fig. 4.

Brevi.-ori was introduced into the obtained p299LYSA to construct a plasmid carrying lysA autonomously replicable in coryneform bacteria. pHK4 was digested with restriction enzymes KpnI and BamHI, and cleaved edges were blunt-ended. Blunt end formation was performed by using DNA Blunting kit (produced by Takara Shuzo) in accordance with a designated method. After the blunt end formation, a phosphorylated KpnI linker (produced by Takara Shuzo) was ligated to make modification so that the DNA fragment corresponding to the Brevi.-ori portion might be excised from pHK4 by digestion with only KpnI. This plasmid was digested with KpnI, and the generated Brevi.-ori DNA fragment was ligated with p299LYSA having been also digested with KpnI to prepare a plasmid containing lysA autonomously replicable in coryneform bacteria. The prepared plasmid was designated as pLYSAB. The process of construction of pLYSAB is shown in Fig. 5.

(2) Determination of nucleotide sequence of lysA from Brevibacterium lactofermentum

Plasmid DNA of p299LYSA was prepared, and its nucleotide sequence was determined in the same manner as described in Example 1. A determined nucleotide sequence and an amino acid sequence deduced to be encoded by the nucleotide sequence are shown in SEQ ID NO: 18. Concerning the nucleotide sequence, an amino acid sequence encoded by argS and an amino acid sequence encoded by lysA are shown in SEQ ID NOs: 19 and 20 respectively.

Example 5: Preparation of aspC from Escherichia coli and Construction of Plasmid Containing aspC

An Escherichia coli JM109 strain was used as a chromosomal DNA donor. Chromosomal DNA was prepared from

the *E. coli* JM109 strain in accordance with an ordinary method. A DNA fragment containing *aspC* was amplified from the chromosomal DNA in accordance with PCR. As for DNA primers used for amplification, synthetic DNA's of 20-mers having nucleotide sequences shown in SEQ ID NOs: 21 and 22 in Sequence Listing respectively were used on the basis of a sequence known for *E. coli* (see Kuramitsu, S. et al., *J. Biochem.*, 97(4), 1259-1262 (1985)). Synthesis of DNA and PCR were performed in the same manner as described in Example 1. The amplified fragment of 1,331 bp was cloned into TA cloning vector pCR1000. The constructed plasmid was designated as pCRASPC.

A nucleotide sequence of the amplified DNA containing *aspC* and an amino acid sequence deduced to be encoded by the nucleotide sequence are shown in SEQ ID NO: 23. Only the amino acid sequence is shown in SEQ ID NO: 24.

Comparative Example 1: Construction of Plasmid Comprising Combination of Mutant *lysC* and *dapA*

A plasmid comprising mutant *lysC*, *dapA*, and replication origin of coryneform bacteria was constructed from the plasmid pCRDAPA comprising *dapA* and the plasmid p399AK9B comprising mutant *lysC* and Brevi.-ori. p399AK9B was completely digested with *Sall*, and then blunt-ended, and was ligated with an *EcoRI* linker to construct a plasmid in which the *Sall* site was modified into an *EcoRI* site. The obtained plasmid was designated as p399AK9BSE. The mutant *lysC* and Brevi.-ori were excised as one fragment by partially digesting p399AK9BSE with *EcoRI*. This fragment was ligated with pCRDAPA having been digested with *EcoRI*. An obtained plasmid was designated as pCRCAB. This plasmid is autonomously replicable in *E. coli* and coryneform bacteria, and it gives kanamycin resistance to a host, the plasmid comprising a combination of mutant *lysC* and *dapA*. The process of construction of pCRCAB is shown in Fig. 6.

Comparative Example 2: Construction of Plasmid Comprising Combination of Mutant *lysC* and *dapB*

A plasmid comprising mutant *lysC* and *dapB* was constructed from the plasmid p399AK9 having mutant *lysC* and the plasmid p399DPR having *dapB*. A fragment of 1,101 bp containing a structural gene of DDPR was extracted by digesting p399DPR with *EcoRV* and *SphI*. This fragment was ligated with p399AK9 having been digested with *Sall* and then blunt-ended and having been further digested with *SphI* to construct a plasmid comprising a combination of mutant *lysC* and *dapB*. This plasmid was designated as p399AKDDPR.

Next, Brevi.-ori was introduced into the obtained p399AKDDPR. The plasmid pHK4 containing Brevi.-ori was digested with a restriction enzyme *KpnI* (produced by Takara Shuzo), and cleaved edges were blunt-ended. Blunt end formation was performed by using DNA Blunting kit (produced by Takara Shuzo) in accordance with a designated method. After the blunt end formation, a phosphorylated *BamHI* linker (produced by Takara Shuzo) was ligated to make modification so that the DNA fragment corresponding to the Brevi.-ori portion might be excised from pHK4 by digestion with only *BamHI*. This plasmid was digested with *BamHI*, and the generated Brevi.-ori DNA fragment was ligated with p399AKDDPR having been also digested with *BamHI* to construct a plasmid containing mutant *lysC* and *dapB* autonomously replicable in coryneform bacteria. The constructed plasmid was designated as pCB. The process of construction of pCB is shown in Fig. 7.

Comparative Example 3: Construction of Plasmid Comprising Combination of *dapA* and *dapB*

The plasmid pCRDAPA comprising *dapA* was digested with *KpnI* and *EcoRI* to extract a DNA fragment containing *dapA*, and was ligated with the vector plasmid pHSG399 having been digested with *KpnI* and *EcoRI*. An obtained plasmid was designated as p399DPS.

On the other hand, the plasmid pCRDAPB comprising *dapB* was digested with *SacII* and *EcoRI* to extract a DNA fragment of 2.0 kb containing a region coding for DDPR, and was ligated with p399DPS having been digested with *SacII* and *EcoRI* to construct a plasmid comprising a combination of *dapA* and *dapB*. The obtained plasmid was designated as p399AB.

Next, Brevi.-ori was introduced into p399AB. pHK4 containing Brevi.-ori was digested with a restriction enzyme *BamHI* (produced by Takara Shuzo), and cleaved edges were blunt-ended. Blunt end formation was performed by using DNA Blunting kit (produced by Takara Shuzo) in accordance with a designated method. After the blunt end formation, a phosphorylated *KpnI* linker (produced by Takara Shuzo) was ligated to make modification so that the DNA fragment corresponding to the Brevi.-ori portion might be excised from pHK4 by digestion with only *KpnI*. This plasmid was digested with *KpnI*, and the generated Brevi.-ori DNA fragment was ligated with p399AB having been also digested with *KpnI* to construct a plasmid containing *dapA* and *dapB* autonomously replicable in coryneform bacteria. The constructed plasmid was designated as pAB. The process of construction of pAB is shown in Fig. 8.

Example 6: Construction of Plasmid Comprising Combination of Mutant *lysC*, *dapA*, and *dapB*

p399DPS was digested with *EcoRI* and *SphI* and blunt-ended, followed by extraction of a *dapA* gene fragment. This

fragment was ligated with the p399AK9 having been digested with Sall and blunt-ended to construct a plasmid p399CA in which mutant lysC and dapA co-existed.

The plasmid pCRDAPB comprising dapB was digested with EcoRI and blunt-ended, followed by digestion with SacI to extract a DNA fragment of 2.0 kb comprising dapB. The plasmid p399CA comprising dapA and mutant lysC was digested with SpeI and blunt-ended, and was thereafter digested with SacI and ligated with the extracted dapB fragment to obtain a plasmid comprising mutant lysC, dapA, and dapB. This plasmid was designated as p399CAB.

Next, Brevi.-ori was introduced into p399CAB. The plasmid pHK4 comprising Brevi.-ori was digested with a restriction enzyme BamHI (produced by Takara Shuzo), and cleaved edges were blunt-ended. Blunt end formation was performed by using DNA Blunting kit (produced by Takara Shuzo) in accordance with a designated method. After the blunt end formation, a phosphorylated KpnI linker (produced by Takara Shuzo) was ligated to make modification so that the DNA fragment corresponding to the Brevi.-ori portion might be excised from pHK4 by digestion with only KpnI. This plasmid was digested with KpnI, and the generated Brevi.-ori DNA fragment was ligated with p399CAB having been also digested with KpnI to construct a plasmid comprising a combination of mutant lysC, dapA, and dapB autonomously replicable in coryneform bacteria. The constructed plasmid was designated as pCAB. The process of construction of pCAB is shown in Fig. 9.

Example 7: Construction of Plasmid Comprising Combination of Mutant lysC, dapA, dapB, and lysA

The plasmid p299LYSA comprising lysA was digested with KpnI and BamHI and blunt-ended, and then a lysA gene fragment was extracted. This fragment was ligated with pCAB having been digested with HpaI (produced by Takara Shuzo) and blunt-ended to construct a plasmid comprising a combination of mutant lysC, dapA, dapB, and lysA autonomously replicable in coryneform bacteria. The constructed plasmid was designated as pCABL. The process of construction of pCABL is shown in Fig. 10. It is noted that the lysA gene fragment is inserted into a HpaI site in a DNA fragment containing the dapB gene in pCABL, however, the HpaI site is located upstream from a promoter for the dapB gene (nucleotide numbers 611 to 616 in SEQ ID NO: 10), and the dapB gene is not decoupled.

Example 8: Construction of Plasmid Comprising aspC

As a vector for introducing aspC into coryneform bacteria, a cloning vector for coryneform bacteria, pVK7 which was newly constructed was used. pVK7 was constructed by ligating pHSG299, a vector for E.coli (Km^r; Takeshita, S. et al., Gene, 61, 63-74 (1987)) with pAM330, a cryptic plasmid for Brevibacterium lactofermentum as described below. pAM330 was prepared from Brevibacterium lactofermentum ATCC 13869 strain. pHSG299 was digested with a restriction enzyme resulting one cleavage site, AvaI (produced by Takara Shuzo), blunt-ended by using T4 DNA polymerase, and ligated with pAM330 having been digested with HindIII (produced by Takara Shuzo) and blunt-ended by using T4 DNA polymerase. Depending on orientation of the inserted pAM330 in pHSG299, the two obtained plasmids were designated as pVK6 and pVK7, and pVK7 was used for the following experiments. pVK7 is autonomously replicable in both of E. coli and Brevibacterium lactofermentum and has a multiple cloning site originating from pHSG299 and lacZ'. The process of construction of pVK6 and pVK7 is shown in Fig. 11.

With the constructed shuttle vector pVK7, aspC was ligated. pCRASPC was digested with a restriction enzyme EcoRI (produced by Takara Shuzo) and ligated with pVK7 having been also digested with EcoRI. Ligation of DNA was performed by using DNA Ligation kit (produced by Takara Shuzo). Among those in which a fragment of aspC was ligated with pVK7, one in which the fragment was inserted in the same orientation as the transcription orientation of lac promoter possessed by pVK7 was designated as pOm. The process of construction of pOm is shown in Fig. 12.

Example 9: Preparation of aspC from Brevibacterium lactofermentum

(1) Preparation of aspC originating from Brevibacterium lactofermentum

An aspartic acid auxotrophic strain 102-7 belonging to the genus Corynebacterium which was deficient in aspC activity (AAT activity) to be aspartic acid auxotrophic (I. Shiio and K. Ujikawa, J. Biochem., 84, 647 (1978)), was transformed by introducing a gene library (International Publication No. WO95/23224) prepared by ligating various fragments of chromosomal DNA of wild type ATCC 13869 strain of Brevibacterium lactofermentum with a vector which functions in cells of bacteria belonging to the genus Corynebacterium. The obtained transformants were collected and washed with distilled water twice. Tens of thousands of the transformants were plated on agar plates of a minimum medium, Medium 10 containing no nitrogen source other than ammonia (I. Shiio and K. Ujikawa, J. Biochem., 84, 647 (1978)) to obtain transformants which restored aspartic acid auxotrophy and showed excellent growth on the plate. Plasmid DNA was recovered from the obtained strain restoring the aspartic acid auxotrophy, and the obtained plasmid was designated as pAC. When the wild type ATCC 13869 strain of Brevibacterium lactofermentum was transformed

with pAC, the aspC activity of the transformant was increased (Table 1). The activity determination was conducted according to a known method (see Sizer, I.W. and Jenkins, W.T., Meth. Enzymol., vol. 5, 677-679 (1962)).

From the results, it was confirmed that the about 2.5 kb fragment of the chromosomal DNA of the ATCC 13869 strain on the plasmid DNA contained aspC of Brevibacterium lactofermentum.

Table 1

Strain/Plasmid	aspC Activity (Relative value)
AJ13869	1.0
AJ13869/pCABL	8.9

(2) Analysis of aspC originating from Brevibacterium lactofermentum

A nucleotide sequence of the 2.5 kb DNA fragment was determined according to the dideoxy method of Sangar et al. (Proc. Natl. Acad. Sci. USA 74, 5463 (1977)). The determined nucleotide sequence was shown in SEQ ID NO: 25. The nucleotide sequence was analyzed by using GENETYX-MAC Version 7.3 program (Software Kaihatsu KK). ORF (Open Reading Frame) search showed two ORFs which overlapped in the opposite orientation as shown in Fig. 13. The ORF of 432 amino acids or 426 amino acids which was encoded in the normal orientation between ATG of nucleotide number of 579 to 881 or 897 to 899 as an initiation codon and TAG of nucleotide number of 2175 to 2177 as a termination codon in the nucleotide sequence shown in SEQ ID NO: 25 was designated as ORF1. The ORF of 393 amino acids which was encoded in the reverse orientation between GTG complementary to CAC of nucleotide number of 2163 to 2165 as an initiation codon and TGA complementary to TCA of nucleotide number of 984 to 986 as a termination codon in the nucleotide sequence shown in SEQ ID NO: 25 was designated as ORF2.

(3) Determination of ORF coding for aspC

A DNA fragment which did not contained the full length of ORF2 and coded for the full length of ORF1 was amplified by PCR from pAC to confirm whether ORF codes for the AAT protein among the two ORFs. As for DNA primers used for amplification, synthetic DNAs of 23-mers having nucleotide sequences shown in SEQ ID NOs: 26 and 27 in Sequence Listing respectively were used on the basis of the sequence shown in SEQ ID NO: 25. Synthesis of DNA and PCR were performed in the same manner as described in Example 1. The amplified fragment of 2,062 bp of the nucleotide number 126 to 2,187 in the nucleotide sequence shown in SEQ ID NO: 25 was cloned into TA cloning vector pCR2.1 (produced by Invitrogen). The constructed plasmid was designated as pCRORF1.

In the same manner, a gene fragment of 1,543 bp of the nucleotide number 975 to 2,517 in the nucleotide sequence shown in SEQ ID NO: 25, which coded for the full length of only ORF2, was amplified and cloned. The constructed plasmid was designated as pCRORF2.

To introduce the cloned DNA fragments into cells of bacteria belonging to the genus Corynebacterium, the DNA fragments were ligated with the shuttle vector described in Example 8. pCRORF1 was digested with a restriction enzyme EcoRI (produced by Takara Shuzo), and ligated with pVK7 having been digested with the restriction enzyme EcoRI. Ligation of DNA was performed by using DNA Ligation kit (produced by Takara Shuzo). The constructed plasmid was designated as pORF1. The process of construction of pORF1 is shown in Fig. 14.

In the same manner, pORF2 was constructed from pCRORF2 and pVK7.

The prepared pORF1 and pORF2 were introduced into cells of Brevibacterium lactofermentum wild type ATCC 13869 strain in the same manner as in Example 9. The aspC activities of ATCC 13869 and obtained plasmid-introduced strains ATCC 13869/pORF1 and ATCC 13869/pORF2 were determined. The activity determination was conducted in the same manner as described in Example 1. As shown in Table 2, an increase in the aspC activity was observed only for ATCC 13869/pORF1, indicating that aspC is encoded by ORF1.

The nucleotide sequence of aspC of Brevibacterium lactofermentum determined by the above-mentioned experiments and an amino acid sequence deduced to be encoded by the nucleotide sequence are shown in SEQ ID NO: 30. Only the amino acid sequence is shown in SEQ ID NO: 31. Homology search on GENE BANK showed no homology to known amino acid sequences including AAT proteins originating from other organisms.

Table 2

Strain/Plasmid	aspC Activity (Relative value)
AJ13869	1.0
AJ13869/pORF1	10.1
AJ13869/pORF2	1.2

Example 10: Introduction of Plasmids Comprising Genes for L-Lysine Biosynthesis into L-Lysine-Producing Bacterium of *Brevibacterium lactofermentum*

The pCABL(Cm^r) constructed in Example 7 was introduced into an L-lysine-producing bacterium AJ11082 (NRRL B-11470) of *Brevibacterium lactofermentum* respectively. The AJ11082 strain has a property of AEC resistance. The plasmid was introduced in accordance with an electric pulse method (Sugimoto et al., Japanese Patent Application Laid-open No. 2-207791). Transformants were selected based on a drug resistance marker possessed by the plasmid. Transformants were selected on a complete medium containing 5 µg/ml of chloramphenicol when a plasmid comprising a chloramphenicol resistance gene was introduced, or transformants were selected on a complete medium containing 25 µg/ml of kanamycin when a plasmid comprising a kanamycin resistance gene was introduced.

The transformant AJ11082/pCABL obtained as described above was transformed with plasmid pOm (Km^r) having aspC of *Escherichia coli* or pORF1 (Km^r) having aspC of *Brevibacterium lactofermentum*. Since pCABL uses pHM1519 as an replication origin in cells of *Brevibacterium lactofermentum* and a Cm resistance gene as a marker, and pOm uses pAM330 as an replication origin in cells of *Brevibacterium lactofermentum* and a Km resistance gene as a marker, both plasmids are stably harbored in cells of *Brevibacterium lactofermentum*. Thus, strains AJ11082/pCABL/pOm and AJ11082/pCABL/pORF1 in which a plasmid containing a gene participating in L-lysine biosynthesis and a plasmid containing aspC were obtained.

In the same manner as described above, p399AK9B(Cm^r), pDPSB(Km^r), pDPRB(Cm^r), pLYSAB(Cm^r), pOm, pCRCAB(Km^r), pAB(Cm^r), pCB(Cm^r), and pCAB(Cm^r) were introduced into the AJ11082 strain to obtain transformants in which mutant *lysC*, *dapA*, *dapB*, *lysA* or *aspC* was enhanced singly, or two or three of these genes were enhanced in combination.

Example 11: Determination of aspC activity of transformants

The aspC activities of the transformants AJ11082/pCABL, AJ11082/pCABL/pOm and AJ11082/pCABL/pORF1 were determined. The activity determination was conducted in the same manner as described in Example 9 (3). As shown in Table 3, it was observed that the lac promoter on the pOm vector also functioned in *Brevibacterium lactofermentum* and the aspC activity of AJ11082/pCABL/pOm increased by about three times. A further increase in the aspC activity by about nine times was observed for AJ11082/pCABL/pORF1.

Table 3

Strain/Plasmid	aspC Activity (Relative value)
AJ11082	1.0
AJ11082/pOm	3.2
AJ11082/pORF1	10.1
AJ11082/pCABL	0.9
AJ11082/pCABL/pOm	2.9
AJ11082/pCABL/pORF1	11.5

Example 12: Production of L-Lysine

Each of the transformants obtained in Example 10 was cultivated in an L-lysine-producing medium to evaluate its L-lysine productivity. The L-lysine-producing medium had the following composition.

[L-Lysine-producing medium]

The following components other than calcium carbonate (in 1 L) were dissolved, and pH was adjusted at 8.0 with KOH. The medium was sterilized at 115°C for 15 minutes, and calcium carbonate (50 g) having been separately sterilized in hot air in a dry state was thereafter added thereto.

Glucose	100 g
(NH ₄) ₂ SO ₄	55 g
KH ₂ PO ₄	1 g
MgSO ₄ · 7H ₂ O	1 g
Biotin	500 µg
Thiamin	2000 µg
FeSO ₄ · 7H ₂ O	0.01 g
MnSO ₄ · 7H ₂ O	0.01 g
Nicotinamide	5 mg
Protein hydrolysate (Mamenou)	30 ml
Calcium carbonate	50 g

Each of the various types of the transformants and the parent strain was inoculated to the medium having the composition described above to perform cultivation at 31.5°C with reciprocating shaking. The amount of produced L-lysine after 40 or 72 hours of cultivation, and the growth after 72 hours (OD₅₆₂) are shown in Table 4. In the table, lysC* represents mutant lysC. The growth was quantitatively determined by measuring OD at 562 nm after 101-fold dilution.

Table 4

Accumulation of L-Lysine after Cultivation for 40 or 72 Hours				
Bacterial strain /plasmid	Introduced gene	Amount of produced L-lysine(g/L)		Growth (OD ₅₆₂ /101)
		after 40hrs	after 72hrs	
AJ11082		22.0	29.8	0.450
AJ11082/p399AK9B	<u>lysC</u> *	16.8	34.5	0.398
AJ11082/pDPSB	<u>dapA</u>	18.7	33.8	0.410
AJ11082/pDPRB	<u>dapB</u>	19.9	29.9	0.445
AJ11082/pLYSAB	<u>lysA</u>	19.8	32.5	0.356
AJ11082/pOm	<u>aspC</u> (E)[Note 1]	21.8	30.9	0.457
AJ11082/pOm	<u>aspC</u> (B)[Note 2]	21.5	31.2	0.450
AJ11082/pCRCAB	<u>lysC</u> *, <u>dapA</u>	19.7	36.5	0.360
AJ11082/pAB	<u>dapA</u> , <u>dapB</u>	19.0	34.8	0.390
AJ11082/pCB	<u>lysC</u> *, <u>dapB</u>	23.3	35.0	0.440
AJ11082/pCAB	<u>lysC</u> *, <u>dapA</u> , <u>dapB</u>	23.0	45.0	0.425
AJ11082/pCABL	<u>lysC</u> *, <u>dapA</u> , <u>dapB</u> , <u>lysA</u>	26.2	46.5	0.379
AJ11082/pCABL/pOm	<u>lysC</u> *, <u>dapA</u> , <u>dapB</u> , <u>lysA</u> , <u>aspC</u> (E)	26.7	47.6	0.415
AJ11082/pCABL/pORF1	<u>lysC</u> *, <u>dapA</u> , <u>dapB</u> , <u>lysA</u> , <u>aspC</u> (B)	27.1	48.8	0.410

Note 1: aspC of Escherichia coliNote 2: aspC of Brevibacterium lactofermentum

As shown in the above, when mutant lysC, dapA, dapB, lysA or aspC was enhanced singly, the amount of produced L-lysine was larger than or equivalent to that produced by the parent strain after 72 hours of cultivation, however, the amount of produced L-lysine was smaller than that produced by the parent strain after 40 hours of cultivation. Namely, the L-lysine-producing speed was lowered in cultivation for a short period. Similarly, when mutant lysC and dapA, or dapA and dapB were enhanced in combination, the amount of produced L-lysine was larger than that produced by the parent strain after 72 hours of cultivation, however, the amount of produced L-lysine was smaller than that produced by the parent strain after 40 hours of cultivation. Thus the L-lysine-producing speed was lowered.

On the contrary, in the case of the strain in which dapB was enhanced together with mutant lysC, the strain in which three of mutant lysC, dapA and dapB were enhanced, and the strain in which four of mutant lysC, dapA, dapB and lysA were enhanced, the accumulated amount of L-lysine was improved in both of the short period and the long period of cultivation.

In the case of the strain in which five of mutant lysC, dapA, dapB, lysA, and aspC of Escherichia coli were enhanced, and the strain in which five of mutant lysC, dapA, dapB, lysA, and aspC of Brevibacterium lactofermentum were enhanced, the L-lysine productivity was further improved in any of the periods. The extent of the improvement of the latter was larger than that of the former.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: AJINOMOTO CO., LTD.
- (ii) TITLE OF INVENTION: METHOD FOR PRODUCING L-LYSINE
- (iii) NUMBER OF SEQUENCES: 31
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE:
 - (B) STREET:
 - (C) CITY:
 - (E) COUNTRY:
 - (F) ZIP:
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: JP 8-325659
 - (B) FILING DATE: 05-DEC-1996
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME:
 - (B) REGISTRATION NUMBER:
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE:
 - (B) TELEFAX:

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 bases
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "synthetic DNA"
- (iv) ANTI-SENSE: no
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:
TCGCGAAGTA GCACCTGTCA CTT

23

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 bases
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "synthetic DNA"
- (iv) ANTI-SENSE: yes
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:
ACGGAATTCA ATCTTACGGC C

21

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1643 bases
 - (B) TYPE: nucleic acid

- (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: genomic DNA
 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: *Brevibacterium lactofermentum*
 (B) STRAIN: ATCC 13869

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

TCGCGAAGTA	GCACCTGTCA	CTTTGTCTC	AAATATTAAA	TCGAATATCA	ATATACGGTC	60
TGTTTATTGG	AACGCATCCC	AGTGGCTGAG	ACGCATCCGC	TAAAGCCCCA	GGAACCTGT	120
GCAGAAAGAA	AACACTCCTC	TGGCTAGGTA	GACACAGTTT	ATAAAGGTAG	AGTTGAGCGG	180
GTAACGTGCA	GCACGTAGAT	CGAAAGGTGC	ACAAAGGTGG	CCCTGGTCGT	ACAGAAATAT	240
GGCGGTTCTT	CGCTTGAGAG	TGCGGAACGC	ATTAGAAACG	TCGCTGAACG	GATCGTTGCC	300
ACCAAGAAGG	CTGGAAATGA	TGTCGTGGTT	GTCTGCTCCG	CAATGGGAGA	CACCACGGAT	360
GAACCTCTAG	AACTTGACAG	GGCAGTGAAT	CCCGTTCCGC	CAGCTCGTGA	AATGGATATG	420
CTCCTGACTG	CTGGTGAGCG	TATTTCTAAC	GCTCTCGTCG	CCATGGCTAT	TGAGTCCCTT	480
GGCGCAGAAG	CTCAATCTTT	CACTGGCTCT	CAGGCTGGTG	TGCTCACCAC	CGAGCGCCAC	540
GGAAACGCAC	GCATTGTTGA	CGTCACACCG	GGTCGTGTGC	GTGAAGCACT	CGATGAGGGC	600
AAGATCTGCA	TTGTTGCTGG	TTTTCAGGGT	GTAAATAAAG	AAACCCGCGA	TGTCACCACG	660
TTGGGTCTGT	GTGGTTCTGA	CACCACTGCA	GTTGCGTTGG	CAGCTGCTTT	GAACGCTGAT	720
GTGTGTGAGA	TTTACTCGGA	CGTTGACGGT	GTGTATACCG	CTGACCCGCG	CATCGTTCCT	780
AATGCACAGA	AGCTGGAAAA	GCTCAGCTTC	GAAGAAATGC	TGGAACCTGC	TGCTGTTGGC	840
TCCAAGATTT	TGGTGCTGCG	CAGTGTGAA	TACGCTCGTG	CATTCAATGT	GCCACTTCGC	900
GTACGCTCGT	CTTATAGTAA	TGATCCCAGC	ACTTTGATTG	CCGGCTCTAT	GGAGGATATT	960
CCTGTGGAAG	AAGCAGTCC	TACCGGTGTC	GCAACCGACA	AGTCCGAAGC	CAAAGTAACC	1020
GTTCTGGGTA	TTTCCGATAA	GCCAGGCGAG	GCTGCCAAGG	TTTCCGTGCG	GTTGGCTGAT	1080
GCAGAAATCA	ACATTGACAT	GGTTCTGCAG	AACGCTCCT	CTGTGGAAGA	CGGCACCACC	1140
GACATCACGT	TCACCTGCC	TCGCGCTGAC	GGACGCCGTG	CGATGGAGAT	CTTGAAGAAG	1200
CTTCAGGTTT	AGGGCAACTG	GACCAATGTG	CTTTACGACG	ACCAGGTCGG	CAAAGTCTCC	1260
CTCGTGGGTG	CTGGCATGAA	GTCTACCCCA	GGTGTTACCG	CAGAGTTCAT	GGAAGCTCTG	1320
CGCGATGTCA	ACGTGAACAT	CGAATTGATT	TCCACCTCTG	AGATCCGCAT	TTCCGTGCTG	1380
ATCCGTGAAG	ATGATCTGGA	TGCTGCTGCA	CGTGCAATTG	ATGAGCAGTT	CCAGCTGGGC	1440
GGCGAAGACG	AAGCCGTCGT	TTATGCAGGC	ACCGGACGCT	AAAGTTTTAA	AGGAGTAGTT	1500
TTACAATGAC	CACCATCGCA	GTTGTTGGTG	CAACCGGCCA	GGTCGGCCAG	GTTATGCGCA	1560
CCCTTTTGGG	AGAGCGCAAT	TTCCAGCTG	ACACTGTTTC	TTTCTTTGCT	TCCCCGCGTT	1620
CCGCAGGCCG	TAAGATTGAA	TTC				1643

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1643 bases
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Brevibacterium lactofermentum*
 (B) STRAIN: ATCC 13869

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 217..1482

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

TCGCGAAGTA	GCACCTGTCA	CTTTGTCTC	AAATATTAAA	TCGAATATCA	ATATACGGTC	60
TGTTTATTGG	AACGCATCCC	AGTGGCTGAG	ACGCATCCGC	TAAAGCCCCA	GGAACCTGT	120
GCAGAAAGAA	AACACTCCTC	TGGCTAGGTA	GACACAGTTT	ATAAAGGTAG	AGTTGAGCGG	180
GTAACGTGCA	GCACGTAGAT	CGAAAGGTGC	ACAAAG	GTG	GCC CTG GTC GTA CAG	234
				Met Ala Leu Val Val Gln		
				1	5	
AAA TAT GGC GGT TCC TCG CTT GAG AGT GCG GAA CGC ATT AGA AAC GTC						282
Lys Tyr Gly Gly Ser Ser Leu Glu Ser Ala Glu Arg Ile Arg Asn Val						

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					10					15				20						
	GCT	GAA	CGG	ATC	GTT	GCC	ACC	AAG	AAG	GCT	GGA	AAT	GAT	GTC	GTG	GTT				330
	Ala	Glu	Arg	Ile	Val	Ala	Thr	Lys	Lys	Ala	Gly	Asn	Asp	Val	Val	Val				
5			25					30					35							
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	Val	Cys	Ser	Ala	Met	Gly	Asp	Thr	Thr	Asp	Glu	Leu	Leu	Glu	Leu	Ala				
		40					45					50								
	GCG	GCA	GTG	AAT	CCC	GTT	CCG	CCA	GCT	CGT	GAA	ATG	GAT	ATG	CTC	CTG				426
10	Ala	Ala	Val	Asn	Pro	Val	Pro	Pro	Ala	Arg	Glu	Met	Asp	Met	Leu	Leu				
		55				60					65				70					
	ACT	GCT	GGT	GAG	CGT	ATT	TCT	AAC	GCT	CTC	GTC	GCC	ATG	GCT	ATT	GAG				474
	Thr	Ala	Gly	Glu	Arg	Ile	Ser	Asn	Ala	Leu	Val	Ala	Met	Ala	Ile	Glu				
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	TCC	CTT	GGC	GCA	GAA	GCT	CAA	TCT	TTC	ACT	GGC	TCT	CAG	GCT	GGT	GTG				522
15	Ser	Leu	Gly	Ala	Glu	Ala	Gln	Ser	Phe	Thr	Gly	Ser	Gln	Ala	Gly	Val				
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	CTC	ACC	ACC	GAG	CGC	CAC	GGA	AAC	GCA	CGC	ATT	GTT	GAC	GTC	ACA	CCG				570
	Leu	Thr	Thr	Glu	Arg	His	Gly	Asn	Ala	Arg	Ile	Val	Asp	Val	Thr	Pro				
			105					110					115							
	GGT	CGT	GTG	CGT	GAA	GCA	CTC	GAT	GAG	GGC	AAG	ATC	TGC	ATT	GTT	GCT				618
20	Gly	Arg	Val	Arg	Glu	Ala	Leu	Asp	Glu	Gly	Lys	Ile	Cys	Ile	Val	Ala				
		120				125				130										
	GGT	TTT	CAG	GGT	GTT	AAT	AAA	GAA	ACC	CGC	GAT	GTC	ACC	ACG	TTG	GGT				666
	Gly	Phe	Gln	Gly	Val	Asn	Lys	Glu	Thr	Arg	Asp	Val	Thr	Thr	Leu	Gly				
		135			140					145					150					
	CGT	GGT	GGT	TCT	GAC	ACC	ACT	GCA	GTT	GCG	TTG	GCA	GCT	GCT	TTG	AAC				714
25	Arg	Gly	Gly	Ser	Asp	Thr	Thr	Ala	Val	Ala	Leu	Ala	Ala	Ala	Leu	Asn				
			155					160							165					
	GCT	GAT	GTG	TGT	GAG	ATT	TAC	TCG	GAC	GTT	GAC	GGT	GTG	TAT	ACC	GCT				762
	Ala	Asp	Val	Cys	Glu	Ile	Tyr	Ser	Asp	Val	Asp	Gly	Val	Tyr	Thr	Ala				
			170					175						180						
	GAC	CCG	CGC	ATC	GTT	CCT	AAT	GCA	CAG	AAG	CTG	GAA	AAG	CTC	AGC	TTC				810
30	Asp	Pro	Arg	Ile	Val	Pro	Asn	Ala	Gln	Lys	Leu	Glu	Lys	Leu	Ser	Phe				
			185				190						195							
	GAA	GAA	ATG	CTG	GAA	CTT	GCT	GCT	GTT	GGC	TCC	AAG	ATT	TTG	GTG	CTG				858
	Glu	Glu	Met	Leu	Glu	Leu	Ala	Ala	Val	Gly	Ser	Lys	Ile	Leu	Val	Leu				
		200				205				210										
	CGC	AGT	GTT	GAA	TAC	GCT	CGT	GCA	TTC	AAT	GTG	CCA	CTT	CGC	GTA	CGC				906
35	Arg	Ser	Val	Glu	Tyr	Ala	Arg	Ala	Phe	Asn	Val	Pro	Leu	Arg	Val	Arg				
			215			220				225					230					
	TCG	TCT	TAT	AGT	AAT	GAT	CCC	GGC	ACT	TTG	ATT	GCC	GGC	TCT	ATG	GAG				954
	Ser	Ser	Tyr	Ser	Asn	Asp	Pro	Gly	Thr	Leu	Ile	Ala	Gly	Ser	Met	Glu				
			235					240						245						
	GAT	ATT	CCT	GTG	GAA	GAA	GCA	GTC	CTT	ACC	GGT	GTC	GCA	ACC	GAC	AAG				1002
40	Asp	Ile	Pro	Val	Glu	Glu	Ala	Val	Leu	Thr	Gly	Val	Ala	Thr	Asp	Lys				
			250				255						260							
	TCC	GAA	GCC	AAA	GTA	ACC	GTT	CTG	GGT	ATT	TCC	GAT	AAG	CCA	GGC	GAG				1050
	Ser	Glu	Ala	Lys	Val	Thr	Val	Leu	Gly	Ile	Ser	Asp	Lys	Pro	Gly	Glu				
		265					270					275								
45	GCT	GCC	AAG	GTT	TTC	CGT	GCG	TTG	GCT	GAT	GCA	GAA	ATC	AAC	ATT	GAC				1098
	Ala	Ala	Lys	Val	Phe	Arg	Ala	Leu	Ala	Asp	Ala	Glu	Ile	Asn	Ile	Asp				
		280				285					290									
	ATG	GTT	CTG	CAG	AAC	GTC	TCC	TCT	GTG	GAA	GAC	GGC	ACC	ACC	GAC	ATC				1146
	Met	Val	Leu	Gln	Asn	Val	Ser	Ser	Val	Glu	Asp	Gly	Thr	Thr	Asp	Ile				
		295			300					305					310					
50	ACG	TTC	ACC	TGC	CCT	CGC	GCT	GAC	GGA	CGC	CGT	GCG	ATG	GAG	ATC	TTG				1194
	Thr	Phe	Thr	Cys	Pro	Arg	Ala	Asp	Gly	Arg	Arg	Ala	Met	Glu	Ile	Leu				
			315					320							325					

AAG AAG CTT CAG GTT CAG GGC AAC TGG ACC AAT GTG CTT TAC GAC GAC 1242
 Lys Lys Leu Gln Val Gln Gly Asn Trp Thr Asn Val Leu Tyr Asp Asp
 330 335 340
 5 CAG GTC GGC AAA GTC TCC CTC GTG GGT GCT GGC ATG AAG TCT CAC CCA 1290
 Gln Val Gly Lys Val Ser Leu Val Gly Ala Gly Met Lys Ser His Pro
 345 350 355
 GGT GTT ACC GCA GAG TTC ATG GAA GCT CTG CGC GAT GTC AAC GTG AAC 1338
 Gly Val Thr Ala Glu Phe Met Glu Ala Leu Arg Asp Val Asn Val Asn
 360 365 370
 10 ATC GAA TTG ATT TCC ACC TCT GAG ATC CGC ATT TCC GTG CTG ATC CGT 1386
 Ile Glu Leu Ile Ser Thr Ser Glu Ile Arg Ile Ser Val Leu Ile Arg
 375 380 385 390
 GAA GAT GAT CTG GAT GCT GCT GCA CGT GCA TTG CAT GAG CAG TTC CAG 1434
 Glu Asp Asp Leu Asp Ala Ala Ala Arg Ala Leu His Glu Gln Phe Gln
 395 400 405
 15 CTG GGC GGC GAA GAC GAA GCC GTC GTT TAT GCA GGC ACC GGA CGC TAA 1482
 Leu Gly Gly Glu Asp Glu Ala Val Val Tyr Ala Gly Thr Gly Arg
 410 415 420
 AGTTTAAAG GAGTAGTTTT ACAATGACCA CCATCGCAGT TGTGTTGCA ACCGGCCAGG 1542
 TCGGCCAGGT TATGCGCACC CTTTGGGAAG AGCGCAATTT CCCAGCTGAC ACTGTTCTGT 1602
 TCTTTGCTTC CCCGCGTTCC GCAGGCCGTA AGATTGAATT C 1643

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 421 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Met Ala Leu Val Val Gln Lys Tyr Gly Gly Ser Ser Leu Glu Ser Ala
 1 5 10 15
 Glu Arg Ile Arg Asn Val Ala Glu Arg Ile Val Ala Thr Lys Lys Ala
 20 25 30
 Gly Asn Asp Val Val Val Val Cys Ser Ala Met Gly Asp Thr Thr Asp
 35 40 45
 Glu Leu Leu Glu Leu Ala Ala Val Asn Pro Val Pro Pro Ala Arg
 50 55 60
 Glu Met Asp Met Leu Leu Thr Ala Gly Glu Arg Ile Ser Asn Ala Leu
 65 70 75 80
 Val Ala Met Ala Ile Glu Ser Leu Gly Ala Glu Ala Gln Ser Phe Thr
 85 90 95
 Gly Ser Gln Ala Gly Val Leu Thr Thr Glu Arg His Gly Asn Ala Arg
 100 105 110
 Ile Val Asp Val Thr Pro Gly Arg Val Arg Glu Ala Leu Asp Glu Gly
 115 120 125
 Lys Ile Cys Ile Val Ala Gly Phe Gln Gly Val Asn Lys Glu Thr Arg
 130 135 140
 Asp Val Thr Thr Leu Gly Arg Gly Gly Ser Asp Thr Thr Ala Val Ala
 145 150 155 160
 45 Leu Ala Ala Ala Leu Asn Ala Asp Val Cys Glu Ile Tyr Ser Asp Val
 165 170 175
 Asp Gly Val Tyr Thr Ala Asp Pro Arg Ile Val Pro Asn Ala Gln Lys
 180 185 190
 Leu Glu Lys Leu Ser Phe Glu Glu Met Leu Glu Leu Ala Ala Val Gly
 195 200 205
 50 Ser Lys Ile Leu Val Leu Arg Ser Val Glu Tyr Ala Arg Ala Phe Asn
 210 215 220
 Val Pro Leu Arg Val Arg Ser Ser Tyr Ser Asn Asp Pro Gly Thr Leu

225 230 235 240
 Ile Ala Gly Ser Met Glu Asp Ile Pro Val Glu Glu Ala Val Leu Thr
 245 250 255
 5 Gly Val Ala Thr Asp Lys Ser Glu Ala Lys Val Thr Val Leu Gly Ile
 260 265 270
 Ser Asp Lys Pro Gly Glu Ala Ala Lys Val Phe Arg Ala Leu Ala Asp
 275 280 285
 Ala Glu Ile Asn Ile Asp Met Val Leu Gln Asn Val Ser Ser Val Glu
 290 295 300
 10 Asp Gly Thr Thr Asp Ile Thr Phe Thr Cys Pro Arg Ala Asp Gly Arg
 305 310 315 320
 Arg Ala Met Glu Ile Leu Lys Lys Leu Gln Val Gln Gly Asn Trp Thr
 325 330 335
 Asn Val Leu Tyr Asp Asp Gln Val Gly Lys Val Ser Leu Val Gly Ala
 340 345 350
 15 Gly Met Lys Ser His Pro Gly Val Thr Ala Glu Phe Met Glu Ala Leu
 355 360 365
 Arg Asp Val Asn Val Asn Ile Glu Leu Ile Ser Thr Ser Glu Ile Arg
 370 375 380
 Ile Ser Val Leu Ile Arg Glu Asp Asp Leu Asp Ala Ala Ala Arg Ala
 385 390 395 400
 20 Leu His Glu Gln Phe Gln Leu Gly Gly Glu Asp Glu Ala Val Val Tyr
 405 410 415
 Ala Gly Thr Gly Arg
 420

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1643 bases
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Brevibacterium lactofermentum*
 (B) STRAIN: ATCC 13869

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 964..1482

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

TCGCGAAGTA	GCACCTGTCA	CTTTTGTCTC	AAATATTAAA	TCGAATATCA	ATATACGGTC	60
TGTTTATTGG	AACGCATCCC	AGTGGCTGAG	ACGCATCCGC	TAAAGCCCCA	GGAACCTGT	120
GCAGAAAGAA	AACACTCCTC	TGGCTAGGTA	GACACAGTTT	ATAAAGGTAG	AGTTGAGCGG	180
GTAACCTGTCA	GCACGTAGAT	CGAAAGGTGC	ACAAAGGTGG	CCCTGGTCGT	ACAGAAATAT	240
GGCGGTTCCCT	CGCTTGAGAG	TGCGGAACGC	ATTAGAAACG	TCGCTGAACG	GATCGTTGCC	300
ACCAAGAAGG	CTGGAAATGA	TGTCGTGGTT	GTCTGCTCCG	CAATGGGAGA	CACCACGGAT	360
GAACCTCTAG	AACTTGCAGC	GGCAGTGAAT	CCCGTTCCGC	CAGCTCGTGA	AATGGATATG	420
CTCCTGACTG	CTGGTGAGCG	TATTTCTAAC	GCTCTCGTCG	CCATGGCTAT	TGAGTCCCTT	480
GGCGCAGAAG	CTCAATCTTT	CACTGGCTCT	CAGGCTGGTG	TGCTCACCAC	CGAGCGCCAC	540
GGAAACGCAC	GCATTGTTGA	CGTCACACCG	GGTCGTGTGC	GTGAAGCACT	CGATGAGGGC	600
AAGATCTGCA	TTGTTGCTGG	TTTTCAGGGT	GTTAATAAAG	AAACCCGCGA	TGTCACCACG	660
TTGGGTCGTG	GTGGTTCTGA	CACCACTGCA	GTTGCGTTGG	CAGCTGCTTT	GAACGCTGAT	720
GTGTGTGAGA	TTTACTCGGA	CGTTGACGGT	GTGTATACCG	CTGACCCGCG	CATCGTTCCT	780
AATGCACAGA	AGCTGGAAAA	GCTCAGCTTC	GAAGAAATGC	TGGAACCTGC	TGCTGTTGGC	840
TCCAGATTTT	TGGTGCTGCG	CAGTGTTGAA	TACGCTCGTG	CATTCAATGT	GCCACTTCGC	900
GTACGCTCGT	CTTATAGTAA	TGATCCCGGC	ACTTTGATTG	CCGGCTCTAT	GGAGGATATT	960
CCT	GTG	GAA	GAA	GCA	GTC	CTT
	ACC	GGT	GTC	GCA	ACC	GAC
	AAG	TCC	GAA			
	Met	Glu	Glu	Ala	Val	Leu
	Thr	Gly	Val	Ala	Thr	Asp
	Lys	Ser	Glu			

1 5 10 15
 GCC AAA GTA ACC GTT CTG GGT ATT TCC GAT AAG CCA GGC GAG GCT GCC 1056
 Ala Lys Val Thr Val Leu Gly Ile Ser Asp Lys Pro Gly Glu Ala Ala
 20 25 30
 5 AAG GTT TTC CGT GCG TTG GCT GAT GCA GAA ATC AAC ATT GAC ATG GTT 1104
 Lys Val Phe Arg Ala Leu Ala Asp Ala Glu Ile Asn Ile Asp Met Val
 35 40 45
 CTG CAG AAC GTC TCC TCT GTG GAA GAC GGC ACC ACC GAC ATC ACG TTC 1152
 Leu Gln Asn Val Ser Ser Val Glu Asp Gly Thr Thr Asp Ile Thr Phe
 50 55 60
 10 ACC TGC CCT CGC GCT GAC GGA CGC CGT GCG ATG GAG ATC TTG AAG AAG 1200
 Thr Cys Pro Arg Ala Asp Gly Arg Arg Ala Met Glu Ile Leu Lys Lys
 65 70 75
 CTT CAG GTT CAG GGC AAC TGG ACC AAT GTG CTT TAC GAC GAC CAG GTC 1248
 Leu Gln Val Gln Gly Asn Trp Thr Asn Val Leu Tyr Asp Asp Gln Val
 80 85 90 95
 15 GGC AAA GTC TCC CTC GTG GGT GCT GGC ATG AAG TCT CAC CCA GGT GTT 1296
 Gly Lys Val Ser Leu Val Gly Ala Gly Met Lys Ser His Pro Gly Val
 100 105 110
 ACC GCA GAG TTC ATG GAA GCT CTG CGC GAT GTC AAC GTG AAC ATC GAA 1344
 Thr Ala Glu Phe Met Glu Ala Leu Arg Asp Val Asn Val Asn Ile Glu
 115 120 125
 20 TTG ATT TCC ACC TCT GAG ATC CGC ATT TCC GTG CTG ATC CGT GAA GAT 1392
 Leu Ile Ser Thr Ser Glu Ile Arg Ile Ser Val Leu Ile Arg Glu Asp
 130 135 140
 25 GAT CTG GAT GCT GCT GCA CGT GCA TTG CAT GAG CAG TTC CAG CTG GGC 1440
 Asp Leu Asp Ala Ala Ala Arg Ala Leu His Glu Gln Phe Gln Leu Gly
 145 150 155
 GGC GAA GAC GAA GCC GTC GTT TAT GCA GGC ACC GGA CGC TAAAGTTTAA 1490
 Gly Glu Asp Glu Ala Val Val Tyr Ala Gly Thr Gly Arg
 160 165 170
 30 AGGAGTAGTT TTACAATGAC CACCATCGCA GTTGTGGTG CAACCGGCCA GGTGCGCCAG 1550
 GTTATGCGCA CCCTTTTGA AGAGCGCAAT TTCCCAGCTG ACACTGTTG TTTCTTTGCT 1610
 TCCCCGCGTT CCGCAGGCCG TAAGATTGAA TTC 1643

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 172 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Met Glu Glu Ala Val Leu Thr Gly Val Ala Thr Asp Lys Ser Glu Ala
 1 5 10 15
 40 Lys Val Thr Val Leu Gly Ile Ser Asp Lys Pro Gly Glu Ala Ala Lys
 20 25 30
 Val Phe Arg Ala Leu Ala Asp Ala Glu Ile Asn Ile Asp Met Val Leu
 35 40 45
 45 Gln Asn Val Ser Ser Val Glu Asp Gly Thr Thr Asp Ile Thr Phe Thr
 50 55 60
 Cys Pro Arg Ala Asp Gly Arg Arg Ala Met Glu Ile Leu Lys Lys Leu
 65 70 75 80
 Gln Val Gln Gly Asn Trp Thr Asn Val Leu Tyr Asp Asp Gln Val Gly
 85 90 95
 50 Lys Val Ser Leu Val Gly Ala Gly Met Lys Ser His Pro Gly Val Thr
 100 105 110
 Ala Glu Phe Met Glu Ala Leu Arg Asp Val Asn Val Asn Ile Glu Leu
 115 120 125

Ile Ser Thr Ser Glu Ile Arg Ile Ser Val Leu Ile Arg Glu Asp Asp
 130 135 140
 Leu Asp Ala Ala Ala Arg Ala Leu His Glu Gln Phe Gln Leu Gly Gly
 145 150 155 160
 Glu Asp Glu Ala Val Tyr Ala Gly Thr Gly Arg
 165 170

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 bases
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA"

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GGATCCCCAA TCGATACCTG GAA

23

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 bases
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA"

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CGGTTTCATCG CCAAGTTTTT CTT

23

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2001 bases
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Brevibacterium lactofermentum

- (B) STRAIN: ATCC 13869

(ix) FEATURE:

- (A) NAME/KEY: CDS

- (B) LOCATION: 730..1473

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GGATCCCCAA TCGATACCTG GAACGACAAC CTGATCAGGA TATCCAATGC CTTGAATATT 60
 GACGTTGAGG AAGGAATCAC CAGCCATCTC AACTGGAAGA CCTGACGCCT GCTGAATTGG 120
 ATCAGTGGCC CAATCGACCC ACCAACCAGG TTGGCTATTA CCGGCGATAT CAAAAACAAC 180
 TCGCGTGAAC GTTTCGTGCT CGGCAACGCG GATGCCAGCG ATCGACATAT CGGAGTCACC 240
 AACTTGAGCC TGCTGCTTCT GATCCATCGA CGGGGAACCC AACGGCGGCA AAGCAGTGGG 300
 GGAAGGGGAG TTGGTGACT CTGAATCAGT GGGCTCTGAA GTGGTAGGCG ACGGGGCAGC 360
 ATCTGAAGGC GTGCGAGTTG TGGTGACCGG GTTAGCGGTT TCAGTTTCTG TCACAACCTGG 420
 AGCAGGACTA GCAGAGGTTG TAGGCGTTGA GCCGCTTCCA TCACAAGCAC TTAAAGTAA 480
 AGAGGCGGAA ACCACAAGCG CCAAGGAACT ACCTGCGGAA CCGGCGGTGA AGGGCAACTT 540
 AAGTCTCATA TTCAAAACAT AGTTCCACCT GTGTGATTAA TCTCCAGAAC GGAACAACT 600
 GATGAACAAT CGTTAACAAC ACAGACCAA ACGGTCAGTT AGGTATGGAT ATCAGCACCT 660
 TCTGAATGGG TACGTCTAGA CTGGTGGGCG TTTGAAAAAC TCTTCGCCCC ACGAAAATGA 720
 AGGAGCATA ATG GGA ATC AAG GTT GGC GTT CTC GGA GCC AAA GGC CGT 768
 Met Gly Ile Lys Val Gly Val Leu Gly Ala Lys Gly Arg

1

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(2) INFORMATION FOR SEQ ID NO:11:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 248 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Met Gly Ile Lys Val Gly Val Leu Gly Ala Lys Gly Arg Val Gly Gln
 1 5 10 15
 Thr Ile Val Ala Val Asn Glu Ser Asp Asp Leu Glu Leu Val Ala
 20 25 30
 Glu Ile Gly Val Asp Asp Asp Leu Ser Leu Leu Val Asp Asn Gly Ala
 35 40 45
 Glu Val Val Val Asp Phe Thr Thr Pro Asn Ala Val Met Gly Asn Leu
 50 55 60
 Glu Phe Cys Ile Asn Asn Gly Ile Ser Ala Val Val Gly Thr Thr Gly
 65 70 75 80
 Phe Asp Asp Ala Arg Leu Glu Gln Val Arg Ala Trp Leu Glu Gly Lys
 85 90 95
 Asp Asn Val Gly Val Leu Ile Ala Pro Asn Phe Ala Ile Ser Ala Val
 100 105 110
 Leu Thr Met Val Phe Ser Lys Gln Ala Ala Arg Phe Phe Glu Ser Ala
 115 120 125
 Glu Val Ile Glu Leu His His Pro Asn Lys Leu Asp Ala Pro Ser Gly
 130 135 140
 Thr Ala Ile His Thr Ala Gln Gly Ile Ala Ala Arg Lys Glu Ala
 145 150 155 160
 Gly Met Asp Ala Gln Pro Asp Ala Thr Glu Gln Ala Leu Glu Gly Ser
 165 170 175
 Arg Gly Ala Ser Val Asp Gly Ile Pro Val His Ala Val Arg Met Ser
 180 185 190
 Gly Met Val Ala His Glu Gln Val Ile Phe Gly Thr Gln Gly Gln Thr
 195 200 205
 Leu Thr Ile Lys Gln Asp Ser Tyr Asp Arg Asn Ser Phe Ala Pro Gly
 210 215 220
 Val Leu Val Gly Val Arg Asn Ile Ala Gln His Pro Gly Leu Val Val
 225 230 235 240
 Gly Leu Glu His Tyr Leu Gly Leu
 245

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 bases
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "synthetic DNA"

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GTCGACGGAT CGCAAATGGC AAC

23

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 bases
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "synthetic DNA"

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:
GGATCCTTGA GCACCTTGCG CAG

23

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1411 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Brevibacterium lactofermentum*
(B) STRAIN: ATCC 13869

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 311..1213

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

CTCTCGATAT	CGAGAGAGAA	GCAGCGCCAC	GGTTTTTCGG	TGATTTTGAG	ATTGAAACTT	60
TGGCAGACGG	ATCGCAAATG	GCAACAAGCC	CGTATGTCAT	GGACTTTTAA	CGCAAAGCTC	120
ACACCCACGA	GCTAAAAATT	CATATAGTTA	AGACAACATT	TTTGGCTGTA	AAAGACAGCC	180
GTAAAAACCT	CTTGCTCATG	TCAATTGTTC	TTATCGGAAT	GTGGCTTGGG	CGATTGTAT	240
GCAAAGTTG	TTAGGTTTTT	TGCGGGGTTG	TTTAACCCCC	AAATGAGGGA	AGAAGGTAAC	300
CTTGAACCT	ATG AGC ACA	GGT TTA ACA	GCT AAG ACC	GGA GTA GAG	CAC	349
	Met Ser Thr	Gly Leu Thr	Ala Lys Thr	Gly Val Glu	His	
	1	5	10			
TTC GGC ACC GTT GGA GTA GCA ATG GTT ACT CCA TTC ACG GAA TCC GGA						397
Phe Gly Thr Val Gly Val Ala Met Val Thr Pro Phe Thr Glu Ser Gly						
	15	20	25			
GAC ATC GAT ATC GCT GCT GGC CGC GAA GTC GCG GCT TAT TTG GTT GAT						445
Asp Ile Asp Ile Ala Ala Gly Arg Glu Val Ala Ala Tyr Leu Val Asp						
	30	35	40	45		
AAG GGC TTG GAT TCT TTG GTT CTC GCG GGC ACC ACT GGT GAA TCC CCA						493
Lys Gly Leu Asp Ser Leu Val Leu Ala Gly Thr Thr Gly Glu Ser Pro						
	50	55	60			
ACG ACA ACC GCC GCT GAA AAA CTA GAA CTG CTC AAG GCC GTT CGT GAG						541
Thr Thr Thr Ala Ala Glu Lys Leu Glu Leu Leu Lys Ala Val Arg Glu						
	65	70	75			
GAA GTT GGG GAT CGG GCG AAC GTC ATC GCC GGT GTC GGA ACC AAC AAC						589
Glu Val Gly Asp Arg Ala Asn Val Ile Ala Gly Val Gly Thr Asn Asn						
	80	85	90			
ACG CGG ACA TCT GTG GAA CTT GCG GAA GCT GCT GCT TCT GCT GGC GCA						637
Thr Arg Thr Ser Val Glu Leu Ala Glu Ala Ala Ala Ser Ala Gly Ala						
	95	100	105			
GAC GGC CTT TTA GTT GTA ACT CCT TAT TAC TCC AAG CCG AGC CAA GAG						685
Asp Gly Leu Leu Val Val Thr Pro Tyr Tyr Ser Lys Pro Ser Gln Glu						
	110	115	120	125		
GGA TTG CTG GCG CAC TTC GGT GCA ATT GCT GCA GCA ACA GAG GTT CCA						733
Gly Leu Leu Ala His Phe Gly Ala Ile Ala Ala Ala Thr Glu Val Pro						
	130	135	140			
ATT TGT CTC TAT GAC ATT CCT GGT CGG TCA GGT ATT CCA ATT GAG TCT						781
Ile Cys Leu Tyr Asp Ile Pro Gly Arg Ser Gly Ile Pro Ile Glu Ser						
	145	150	155			
GAT ACC ATG AGA CGC CTG AGT GAA TTA CCT ACG ATT TTG GCG GTC AAG						829
Asp Thr Met Arg Arg Leu Ser Glu Leu Pro Thr Ile Leu Ala Val Lys						
	160	165	170			
GAC GCC AAG GGT GAC CTC GTT GCA GCC ACG TCA TTG ATC AAA GAA ACG						877
Asp Ala Lys Gly Asp Leu Val Ala Ala Thr Ser Leu Ile Lys Glu Thr						
	175	180	185			

5 GGA CTT GCC TGG TAT TCA GGC GAT GAC CCA CTA AAC CTT GTT TGG CTT 925
 Gly Leu Ala Trp Tyr Ser Gly Asp Asp Pro Leu Asn Leu Val Trp Leu
 190 195 200 205
 GCT TTG GGC GGA TCA GGT TTC ATT TCC GTA ATT GGA CAT GCA GCC CCC 973
 Ala Leu Gly Gly Ser Gly Phe Ile Ser Val Ile Gly His Ala Ala Pro
 210 215 220
 ACA GCA TTA CGT GAG TTG TAC ACA AGC TTC GAG GAA GGC GAC CTC GTC 1021
 Thr Ala Leu Arg Glu Leu Tyr Thr Ser Phe Glu Glu Gly Asp Leu Val
 225 230 235
 10 CGT GCG CGG GAA ATC AAC GCC AAA CTA TCA CCG CTG GTA GCT GCC CAA 1069
 Arg Ala Arg Glu Ile Asn Ala Lys Leu Ser Pro Leu Val Ala Ala Gln
 240 245 250
 GGT CGC TTG GGT GGA GTC AGC TTG GCA AAA GCT GCT CTG CGT CTG CAG 1117
 Gly Arg Leu Gly Gly Val Ser Leu Ala Lys Ala Ala Leu Arg Leu Gln
 255 260 265
 15 GGC ATC AAC GTA GGA GAT CCT CGA CTT CCA ATT ATG GCT CCA AAT GAG 1165
 Gly Ile Asn Val Gly Asp Pro Arg Leu Pro Ile Met Ala Pro Asn Glu
 270 275 280 285
 CAG GAA CTT GAG GCT CTC CGA GAA GAC ATG AAA AAA GCT GGA GTT CTA 1213
 Gln Glu Leu Glu Ala Leu Arg Glu Asp Met Lys Lys Ala Gly Val Leu
 290 295 300
 20 TAAATATGAA TGATTCCCGA AATCGCGGCC GGAAGGTTAC CCGCAAGGCG GCCCACCAGA 1273
 AGCTGGTCAG GAAAACCATC TGATACCCC TGTCTTTCAG GCACCAGATG CTTCCTCTAA 1333
 CCAGAGCGCT GTAAAAGCTG AGACCGCCGG AAACGACAAAT CGGGATGCTG CGCAAGGTGC 1393
 TCAAGGATCC CAACATTC 1411

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 301 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

30 Met Ser Thr Gly Leu Thr Ala Lys Thr Gly Val Glu His Phe Gly Thr
 1 5 10 15
 Val Gly Val Ala Met Val Thr Pro Phe Thr Glu Ser Gly Asp Ile Asp
 20 25 30
 35 Ile Ala Ala Gly Arg Glu Val Ala Tyr Leu Val Asp Lys Gly Leu
 35 40 45
 Asp Ser Leu Val Leu Ala Gly Thr Thr Gly Glu Ser Pro Thr Thr Thr
 50 55 60
 Ala Ala Glu Lys Leu Glu Leu Lys Ala Val Arg Glu Glu Val Gly
 65 70 75 80
 40 Asp Arg Ala Asn Val Ile Ala Gly Val Gly Thr Asn Asn Thr Arg Thr
 85 90 95
 Ser Val Glu Leu Ala Glu Ala Ala Ala Ser Ala Gly Ala Asp Gly Leu
 100 105 110
 Leu Val Val Thr Pro Tyr Tyr Ser Lys Pro Ser Gln Glu Gly Leu Leu
 115 120 125
 45 Ala His Phe Gly Ala Ile Ala Ala Ala Thr Glu Val Pro Ile Cys Leu
 130 135 140
 Tyr Asp Ile Pro Gly Arg Ser Gly Ile Pro Ile Glu Ser Asp Thr Met
 145 150 155 160
 Arg Arg Leu Ser Glu Leu Pro Thr Ile Leu Ala Val Lys Asp Ala Lys
 165 170 175
 50 Gly Asp Leu Val Ala Ala Thr Ser Leu Ile Lys Glu Thr Gly Leu Ala
 180 185 190
 Trp Tyr Ser Gly Asp Asp Pro Leu Asn Leu Val Trp Leu Ala Leu Gly

195 200 205
 Gly Ser Gly Phe Ile Ser Val Ile Gly His Ala Ala Pro Thr Ala Leu
 210 215 220
 Arg Glu Leu Tyr Thr Ser Phe Glu Glu Gly Asp Leu Val Arg Ala Arg
 225 230 235 240
 Glu Ile Asn Ala Lys Leu Ser Pro Leu Val Ala Ala Gln Gly Arg Leu
 245 250 255
 Gly Gly Val Ser Leu Ala Lys Ala Ala Leu Arg Leu Gln Gly Ile Asn
 260 265 270
 Val Gly Asp Pro Arg Leu Pro Ile Met Ala Pro Asn Glu Gln Glu Leu
 275 280 285
 Glu Ala Leu Arg Glu Asp Met Lys Lys Ala Gly Val Leu
 290 295 300

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 bases
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA"

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GTGGAGCCGA CCATTCCGCG AGG

23

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 bases
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA"

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

CCAAARCCGC CCTCCACGGC GAA

23

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3579 bases
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Brevibacterium lactofermentum
 (B) STRAIN: ATCC 13869

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 533..2182

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 2188..3522

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

GTGGAGCCGA CCATTCCGCG AGGCTGCACT GCAACGAGGT CGTAGTTTGT GTACATGGCT 60
 TCTGGCCAGT TCATGGATTG GCTGCCGAAG AAGCTATAGG CATCGCACCA GGGCCACCGA 120
 GTTACCGAAG ATGGTGCCGT GCTTTTCGCC TTGGGCAGGG ACCTTGACAA AGCCCACGCT 180
 GATATCGCCA AGTGAGGGAT CAGAATAGTG CATGGGCACG TCGATGCTGC CACATTGAGC 240

	GGAGGGCAATA TCTACCTGAG GTGGGCATTC TTCCAGCGG ATGTTTCTT GCGCTGCTGC	300
	AGTGGGCATT GATACCAAAA AGGGGCTAAG CGCAGTCGAG GCGGCAAGAA CTGCTACTAC	360
5	CCTTTTATT GTCGAACGGG GCATTACGGC TCCAAGGACG TTTGTTTCT GGGTCAGTTA	420
	CCCCAAAAG CATATACAGA GACCAATGAT TTTTCATTAA AAAGGCAGGG ATTTGTTATA	480
	AGTATGGGTC GTATTCTGTG CGACGGGTGT ACCTCGGCTA GAATTTCTCC CC ATG	535
	Met	
	1	
	ACA CCA GCT GAT CTC GCA ACA TTG ATT AAA GAG ACC GCG GTA GAG GTT	583
10	Thr Pro Ala Asp Leu Ala Thr Leu Ile Lys Glu Thr Ala Val Glu Val	
	5 10 15	
	TTG ACC TCC CGC GAG CTC GAT ACT TCT GTT CTT CCG GAG CAG GTA GTT	631
	Leu Thr Ser Arg Glu Leu Asp Thr Ser Val Leu Pro Glu Gln Val Val	
	20 25 30	
15	GTG GAG CGT CCG CGT AAC CCA GAG CAC GGC GAT TAC GCC ACC AAC ATT	679
	Val Glu Arg Pro Arg Asn Pro Glu His Gly Asp Tyr Ala Thr Asn Ile	
	35 40 45	
	GCA TTG CAG GTG GCT AAA AAG GTC GGT CAG AAC CCT CGG GAT TTG GCT	727
	Ala Leu Gln Val Ala Lys Lys Val Gly Gln Asn Pro Arg Asp Leu Ala	
	50 55 60 65	
20	ACC TGG CTG GCA GAG GCA TTG GCT GCA GAT GAC GCC ATT GAT TCT GCT	775
	Thr Trp Leu Ala Glu Ala Leu Ala Ala Asp Asp Ala Ile Asp Ser Ala	
	70 75 80	
	GAA ATT GCT GGC CCA GGC TTT TTG AAC ATT CGC CTT GCT GCA GCA GCA	823
	Glu Ile Ala Gly Pro Gly Phe Leu Asn Ile Arg Leu Ala Ala Ala Ala	
	85 90 95	
25	CAG GGT GAA ATT GTG GCC AAG ATT CTG GCA CAG GGC GAG ACT TTC GGA	871
	Gln Gly Glu Ile Val Ala Lys Ile Leu Ala Gln Gly Glu Thr Phe Gly	
	100 105 110	
	AAC TCC GAT CAC CTT TCC CAC TTG GAC GTG AAC CTC GAG TTC GTT TCT	919
	Asn Ser Asp His Leu Ser His Leu Asp Val Asn Leu Glu Phe Val Ser	
	115 120 125	
30	GCA AAC CCA ACC GGA CCT ATT CAC CTT GGC GGA ACC CGC TGG GCT GCC	967
	Ala Asn Pro Thr Gly Pro Ile His Leu Gly Gly Thr Arg Trp Ala Ala	
	130 135 140 145	
	GTG GGT GAC TCT TTG GGT CGT GTG CTG GAG GCT TCC GGC GCG AAA GTG	1015
	Val Gly Asp Ser Leu Gly Arg Val Leu Glu Ala Ser Gly Ala Lys Val	
	150 155 160	
35	ACC CGC GAA TAC TAC TTC AAC GAT CAC GGT CGC CAG ATC GAT CGT TTC	1063
	Thr Arg Glu Tyr Tyr Phe Asn Asp His Gly Arg Gln Ile Asp Arg Phe	
	165 170 175	
	GCT TTG TCC CTT CTT GCA GCG GCG AAG GGC GAG CCA ACG CCA GAA GAC	1111
	Ala Leu Ser Leu Leu Ala Ala Ala Lys Gly Glu Pro Thr Pro Glu Asp	
	180 185 190	
40	GGT TAT GGC GGC GAA TAC ATT AAG GAA ATT GCG GAG GCA ATC GTC GAA	1159
	Gly Tyr Gly Gly Glu Tyr Ile Lys Glu Ile Ala Glu Ala Ile Val Glu	
	195 200 205	
	AAG CAT CCT GAA GCG TTG GCT TTG GAG CCT GCC GCA ACC CAG GAG CTT	1207
	Lys His Pro Glu Ala Leu Ala Leu Glu Pro Ala Ala Thr Gln Glu Leu	
	210 215 220 225	
45	TTC CGC GCT GAA GGC GTG GAG ATG ATG TTC GAG CAC ATC AAA TCT TCC	1255
	Phe Arg Ala Glu Gly Val Glu Met Met Phe Glu His Ile Lys Ser Ser	
	230 235 240	
	CTG CAT GAG TTC GGC ACC GAT TTC GAT GTC TAC TAC CAC GAG AAC TCC	1303
	Leu His Glu Phe Gly Thr Asp Phe Asp Val Tyr Tyr His Glu Asn Ser	
	245 250 255	
50	CTG TTC GAG TCC GGT GCG GTG GAC AAG GCC GTG CAG GTG CTG AAG GAC	1351
	Leu Phe Glu Ser Gly Ala Val Asp Lys Ala Val Gln Val Leu Lys Asp	
	260 265 270	

	AAC GGC AAC CTG TAC GAA AAC GAG GGC GCT TGG TGG CTG CGT TCC ACC	1399
	Asn Gly Asn Leu Tyr Glu Asn Glu Gly Ala Trp Trp Leu Arg Ser Thr	
	275 280 285	
5	GAA TTC GGC GAT GAC AAA GAC CGC GTG GTG ATC AAG TCT GAC GGC GAC	1447
	Glu Phe Gly Asp Asp Lys Asp Arg Val Val Ile Lys Ser Asp Gly Asp	
	290 295 300 305	
	GCA GCC TAC ATC GCT GGC GAT ATC GCG TAC GTG GCT GAT AAG TTC TCC	1495
	Ala Ala Tyr Ile Ala Gly Asp Ile Ala Tyr Val Ala Asp Lys Phe Ser	
	310 315 320	
10	CGC GGA CAC AAC CTA AAC ATC TAC ATG TTG GGT GCT GAC CAC CAT GGT	1543
	Arg Gly His Asn Leu Asn Ile Tyr Met Leu Gly Ala Asp His His Gly	
	325 330 335	
	TAC ATC GCG CGC CTG AAG GCA GCG GCG GCG GCA CTT GGC TAC AAG CCA	1591
	Tyr Ile Ala Arg Leu Lys Ala Ala Ala Leu Gly Tyr Lys Pro	
	340 345 350	
15	GAA GGC GTT GAA GTC CTG ATT GGC CAG ATG GTG AAC CTG CTT CGC GAC	1639
	Glu Gly Val Glu Val Leu Ile Gly Gln Met Val Asn Leu Leu Arg Asp	
	355 360 365	
	GGC AAG GCA GTG CGT ATG TCC AAG CGT GCA GGC ACC GTG GTC ACC CTA	1687
	Gly Lys Ala Val Arg Met Ser Lys Arg Ala Gly Thr Val Val Thr Leu	
	370 375 380 385	
20	GAT GAC CTC GTT GAA GCA ATC GGC ATC GAT GCG GCG CGT TAC TCC CTG	1735
	Asp Asp Leu Val Glu Ala Ile Gly Ile Asp Ala Ala Arg Tyr Ser Leu	
	390 395 400	
	ATC CGT TCC TCC GTG GAT TCT TCC CTG GAT ATC GAT CTC GGC CTG TGG	1783
	Ile Arg Ser Ser Val Asp Ser Ser Leu Asp Ile Asp Leu Gly Leu Trp	
	405 410 415	
25	GAA TCC CAG TCC TCC GAC AAC CCT GTG TAC TAC GTG CAG TAC GGA CAC	1831
	Glu Ser Gln Ser Ser Asp Asn Pro Val Tyr Tyr Val Gln Tyr Gly His	
	420 425 430	
	GCT CGT CTG TGC TCC ATC GCG CGC AAG GCA GAG ACC TTG GGT GTC ACC	1879
	Ala Arg Leu Cys Ser Ile Ala Arg Lys Ala Glu Thr Leu Gly Val Thr	
	435 440 445	
30	GAG GAA GGC GCA GAC CTA TCT CTA CTG ACC CAC GAC CGC GAA GGC GAT	1927
	Glu Glu Gly Ala Asp Leu Ser Leu Leu Thr His Asp Arg Glu Gly Asp	
	450 455 460 465	
	CTC ATC CGC ACA CTC GGA GAG TTC CCA GCA GTG GTG AAG GCT GCC GCT	1975
	Leu Ile Arg Thr Leu Gly Glu Phe Pro Ala Val Val Lys Ala Ala Ala	
	470 475 480	
35	GAC CTA CGT GAA CCA CAC CGC ATT GCC CGC TAT GCT GAG GAA TTA GCT	2023
	Asp Leu Arg Glu Pro His Arg Ile Ala Arg Tyr Ala Glu Glu Leu Ala	
	485 490 495	
	GGA ACT TTC CAC CGC TTC TAC GAT TCC TGC CAC ATC CTT CCA AAG GTT	2071
	Gly Thr Phe His Arg Phe Tyr Asp Ser Cys His Ile Leu Pro Lys Val	
	500 505 510	
40	GAT GAG GAT ACG GCA CCA ATC CAC ACA GCA CGT CTG GCA CTT GCA GCA	2119
	Asp Glu Asp Thr Ala Pro Ile His Thr Ala Arg Leu Ala Leu Ala Ala	
	515 520 525	
	GCA ACC CGC CAG ACC CTC GCT AAC GCC CTG CAC CTG GTT GGC GTT TCC	2167
	Ala Thr Arg Gln Thr Leu Ala Asn Ala Leu His Leu Val Gly Val Ser	
	530 535 540 545	
45	GCA CCG GAG AAG ATG TAACA ATG GCT ACA GTT GAA AAT TTC AAT GAA	2214
	Ala Pro Glu Lys Met Met Ala Thr Val Glu Asn Phe Asn Glu	
	550 1 5	
	CTT CCC GCA CAC GTA TGG CCA CGC AAT GCC GTG CGC CAA GAA GAC GGC	2262
	Leu Pro Ala His Val Trp Pro Arg Asn Ala Val Arg Gln Glu Asp Gly	
	10 15 20 25	
50	GTT GTC ACC GTC GCT GGT GTG CCT CTG CCT GAC CTC GCT GAA GAA TAC	2310

EP 0 854 189 A2

	Val	Val	Thr	Val	Ala	Gly	Val	Pro	Leu	Pro	Asp	Leu	Ala	Glu	Glu	Tyr	
					30					35					40		
5	GGA	ACC	CCA	CTG	TTC	GTA	GTC	GAC	GAG	GAC	GAT	TTC	CGT	TCC	CGC	TGT	2358
	Gly	Thr	Pro	Leu	Phe	Val	Val	Asp	Glu	Asp	Asp	Phe	Arg	Ser	Arg	Cys	
				45					50					55			
	CGC	GAC	ATG	GCT	ACC	GCA	TTC	GGT	GGA	CCA	GGC	AAT	GTG	CAC	TAC	GCA	2406
	Arg	Asp	Met	Ala	Thr	Ala	Phe	Gly	Gly	Pro	Gly	Asn	Val	His	Tyr	Ala	
			60					65					70				
10	TCT	AAA	GCG	TTC	CTG	ACC	AAG	ACC	ATT	GCA	CGT	TGG	GTT	GAT	GAA	GAG	2454
	Ser	Lys	Ala	Phe	Leu	Thr	Lys	Thr	Ile	Ala	Arg	Trp	Val	Asp	Glu	Glu	
		75					80					85					
	GGG	CTG	GCA	CTG	GAC	ATT	GCA	TCC	ATC	AAC	GAA	CTG	GGC	ATT	GCC	CTG	2502
	Gly	Leu	Ala	Leu	Asp	Ile	Ala	Ser	Ile	Asn	Glu	Leu	Gly	Ile	Ala	Leu	
		90				95					100				105		
15	GCC	GCT	GGT	TTC	CCC	GCC	AGC	CGT	ATC	ACC	GCG	CAC	GGC	AAC	AAC	AAA	2550
	Ala	Ala	Gly	Phe	Pro	Ala	Ser	Arg	Ile	Thr	Ala	His	Gly	Asn	Asn	Lys	
					110						115				120		
	GGC	GTA	GAG	TTC	CTG	CGC	GCG	TTG	GTT	CAA	AAC	GGT	GTG	GGA	CAC	GTG	2598
	Gly	Val	Glu	Phe	Leu	Arg	Ala	Leu	Val	Gln	Asn	Gly	Val	Gly	His	Val	
				125					130					135			
20	GTG	CTG	GAC	TCC	GCA	CAG	GAA	CTA	GAA	CTG	TTG	GAT	TAC	GTT	GCC	GCT	2646
	Val	Leu	Asp	Ser	Ala	Gln	Glu	Glu	Leu	Leu	Leu	Asp	Tyr	Val	Ala	Ala	
			140					145					150				
	GGT	GAA	GGC	AAG	ATT	CAG	GAC	GTG	TTG	ATC	CGC	GTA	AAG	CCA	GGC	ATC	2694
	Gly	Glu	Gly	Lys	Ile	Gln	Asp	Val	Leu	Ile	Arg	Val	Lys	Pro	Gly	Ile	
		155					160					165					
25	GAA	GCA	CAC	ACC	CAC	GAG	TTC	ATC	GCC	ACT	AGC	CAC	GAA	GAC	CAG	AAG	2742
	Glu	Ala	His	Thr	His	Glu	Phe	Ile	Ala	Thr	Ser	His	Glu	Asp	Gln	Lys	
		170					175				180					185	
	TTC	GGA	TTC	TCC	CTG	GCA	TCC	GGT	TCC	GCA	TTC	GAA	GCA	GCA	AAA	GCC	2790
	Phe	Gly	Phe	Ser	Leu	Ala	Ser	Gly	Ser	Ala	Phe	Glu	Ala	Ala	Lys	Ala	
					190						195				200		
30	GCC	AAC	AAC	GCA	GAA	AAC	CTG	AAC	CTG	GTT	GGC	CTG	CAC	TGC	CAC	GTT	2838
	Ala	Asn	Asn	Ala	Glu	Asn	Leu	Asn	Leu	Val	Gly	Leu	His	Cys	His	Val	
				205					210					215			
	GGT	TCC	CAG	GTG	TTC	GAC	GCC	GAA	GGC	TTC	AAG	CTG	GCA	GCA	GAA	CGC	2886
	Gly	Ser	Gln	Val	Phe	Asp	Ala	Glu	Gly	Phe	Lys	Leu	Ala	Ala	Glu	Arg	
			220					225					230				
35	GTG	TTG	GGC	CTG	TAC	TCA	CAG	ATC	CAC	AGC	GAA	CTG	GGC	GTT	GCC	CTT	2934
	Val	Leu	Gly	Leu	Tyr	Ser	Gln	Ile	His	Ser	Glu	Leu	Gly	Val	Ala	Leu	
		235					240					245					
	CCT	GAA	CTG	GAT	CTC	GGT	GGC	GGA	TAC	GGC	ATT	GCC	TAT	ACC	GCA	GCT	2982
	Pro	Glu	Leu	Asp	Leu	Gly	Gly	Gly	Tyr	Gly	Ile	Ala	Tyr	Thr	Ala	Ala	
40					250		255				260					265	
	GAA	GAA	CCA	CTC	AAC	GTC	GCA	GAA	GTT	GCC	TCC	GAC	CTG	CTC	ACC	GCA	3030
	Glu	Glu	Pro	Leu	Asn	Val	Ala	Glu	Val	Ala	Ser	Asp	Leu	Leu	Thr	Ala	
					270					275					280		
	GTC	GGA	AAA	ATG	GCA	GCG	GAA	CTA	GGC	ATC	GAC	GCA	CCA	ACC	GTG	CTT	3078
	Val	Gly	Lys	Met	Ala	Ala	Glu	Leu	Gly	Ile	Asp	Ala	Pro	Thr	Val	Leu	
45				285					290					295			
	GTT	GAG	CCC	GGC	CGC	GCT	ATC	GCA	GGC	CCC	TCC	ACC	GTG	ACC	ATC	TAC	3126
	Val	Glu	Pro	Gly	Arg	Ala	Ile	Ala	Gly	Pro	Ser	Thr	Val	Thr	Ile	Tyr	
			300					305					310				
	GAA	GTC	GGC	ACC	ACC	AAA	GAC	GTC	CAC	GTA	GAC	GAC	AAA	ACC	CGC		3174
50	Glu	Val	Gly	Thr	Thr	Lys	Asp	Val	His	Val	Asp	Asp	Asp	Lys	Thr	Arg	
		315					320					325					
	CGT	TAC	ATC	GCC	GTG	GAC	GGA	GGC	ATG	TCC	GAC	AAC	ATC	CGC	CCA	GCA	3222
	Arg	Tyr	Ile	Ala	Val	Asp	Gly	Gly	Met	Ser	Asp	Asn	Ile	Arg	Pro	Ala	

330 335 340 345
 CTC TAC GGC TCC GAA TAC GAC GCC CGC GTA GTA TCC CGC TTC GCC GAA 3270
 Leu Tyr Gly Ser Glu Tyr Asp Ala Arg Val Val Ser Arg Phe Ala Glu
 5 350 355 360
 GGA GAC CCA GTA AGC ACC CGC ATC GTG GGC TCC CAC TGC GAA TCC GGC 3318
 Gly Asp Pro Val Ser Thr Arg Ile Val Gly Ser His Cys Glu Ser Gly
 365 370 375
 GAT ATC CTG ATC AAC GAT GAA ATC TAC CCA TCT GAC ATC ACC AGC GGC 3366
 Asp Ile Leu Ile Asn Asp Glu Ile Tyr Pro Ser Asp Ile Thr Ser Gly
 10 380 385 390
 GAC TTC CTT GCA CTC GCA GCC ACC GGC GCA TAC TGC TAC GCC ATG AGC 3414
 Asp Phe Leu Ala Leu Ala Thr Gly Ala Tyr Cys Tyr Ala Met Ser
 395 400 405
 TCC CGC TAC AAC GCC TTC ACA CGG CCC GCC GTC GTG TCC GTC CGC GCT 3462
 Ser Arg Tyr Asn Ala Phe Thr Arg Pro Ala Val Val Ser Val Arg Ala
 15 410 415 420 425
 GGC AGC TCC CGC CTC ATG CTG CGC CGC GAA ACG CTC GAC GAC ATC CTC 3510
 Gly Ser Ser Arg Leu Met Leu Arg Arg Glu Thr Leu Asp Asp Ile Leu
 430 435 440
 TCA CTA GAG GCA TAACGCTTTT CGACGCCTGA CCCC GCCCTT CACCTTCGCC 3562
 Ser Leu Glu Ala
 20 445
 GTGGAGGGCG GTTTTGG 3579

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 550 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Met Thr Pro Ala Asp Leu Ala Thr Leu Ile Lys Glu Thr Ala Val Glu
 1 5 10 15
 Val Leu Thr Ser Arg Glu Leu Asp Thr Ser Val Leu Pro Glu Gln Val
 20 25 30
 Val Val Glu Arg Pro Arg Asn Pro Glu His Gly Asp Tyr Ala Thr Asn
 35 35 40 45
 Ile Ala Leu Gln Val Ala Lys Lys Val Gly Gln Asn Pro Arg Asp Leu
 50 55 60
 Ala Thr Trp Leu Ala Glu Ala Leu Ala Ala Asp Ala Ile Asp Ser
 65 70 75 80
 Ala Glu Ile Ala Gly Pro Gly Phe Leu Asn Ile Arg Leu Ala Ala Ala
 85 90 95
 Ala Gln Gly Glu Ile Val Ala Lys Ile Leu Ala Gln Gly Glu Thr Phe
 100 105 110
 Gly Asn Ser Asp His Leu Ser His Leu Asp Val Asn Leu Glu Phe Val
 115 120 125
 Ser Ala Asn Pro Thr Gly Pro Ile His Leu Gly Gly Thr Arg Trp Ala
 130 135 140
 Ala Val Gly Asp Ser Leu Gly Arg Val Leu Glu Ala Ser Gly Ala Lys
 45 145 150 155 160
 Val Thr Arg Glu Tyr Tyr Phe Asn Asp His Gly Arg Gln Ile Asp Arg
 165 170 175
 Phe Ala Leu Ser Leu Leu Ala Ala Lys Gly Glu Pro Thr Pro Glu
 180 185 190
 Asp Gly Tyr Gly Glu Tyr Ile Lys Glu Ile Ala Glu Ala Ile Val
 50 195 200 205
 Glu Lys His Pro Glu Ala Leu Ala Leu Glu Pro Ala Ala Thr Gln Glu

210 215 220
 Leu Phe Arg Ala Glu Gly Val Glu Met Met Phe Glu His Ile Lys Ser
 225 230 235
 Ser Leu His Glu Phe Gly Thr Asp Phe Asp Val Tyr Tyr His Glu Asn
 245 250 255
 Ser Leu Phe Glu Ser Gly Ala Val Asp Lys Ala Val Gln Val Leu Lys
 260 265 270
 Asp Asn Gly Asn Leu Tyr Glu Asn Glu Gly Ala Trp Trp Leu Arg Ser
 275 280 285
 Thr Glu Phe Gly Asp Asp Lys Asp Arg Val Val Ile Lys Ser Asp Gly
 290 295 300
 Asp Ala Ala Tyr Ile Ala Gly Asp Ile Ala Tyr Val Ala Asp Lys Phe
 305 310 315
 Ser Arg Gly His Asn Leu Asn Ile Tyr Met Leu Gly Ala Asp His His
 325 330 335
 Gly Tyr Ile Ala Arg Leu Lys Ala Ala Ala Ala Leu Gly Tyr Lys
 340 345 350
 Pro Glu Gly Val Glu Val Leu Ile Gly Gln Met Val Asn Leu Leu Arg
 355 360 365
 Asp Gly Lys Ala Val Arg Met Ser Lys Arg Ala Gly Thr Val Val Thr
 370 375 380
 Leu Asp Asp Leu Val Glu Ala Ile Gly Ile Asp Ala Ala Arg Tyr Ser
 385 390 395
 Leu Ile Arg Ser Ser Val Asp Ser Ser Leu Asp Ile Asp Leu Gly Leu
 405 410 415
 Trp Glu Ser Gln Ser Ser Asp Asn Pro Val Tyr Tyr Val Gln Tyr Gly
 420 425 430
 His Ala Arg Leu Cys Ser Ile Ala Arg Lys Ala Glu Thr Leu Gly Val
 435 440 445
 Thr Glu Glu Gly Ala Asp Leu Ser Leu Leu Thr His Asp Arg Glu Gly
 450 455 460
 Asp Leu Ile Arg Thr Leu Gly Glu Phe Pro Ala Val Val Lys Ala Ala
 465 470 475
 Ala Asp Leu Arg Glu Pro His Arg Ile Ala Arg Tyr Ala Glu Glu Leu
 485 490 495
 Ala Gly Thr Phe His Arg Phe Tyr Asp Ser Cys His Ile Leu Pro Lys
 500 505 510
 Val Asp Glu Asp Thr Ala Pro Ile His Thr Ala Arg Leu Ala Leu Ala
 515 520 525
 Ala Ala Thr Arg Gln Thr Leu Ala Asn Ala Leu His Leu Val Gly Val
 530 535 540
 Ser Ala Pro Glu Lys Met
 545 550

 (2) INFORMATION FOR SEQ ID NO:20:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 445 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: protein
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:
 Met Ala Thr Val Glu Asn Phe Asn Glu Leu Pro Ala His Val Trp Pro
 1 5 10 15
 Arg Asn Ala Val Arg Gln Glu Asp Gly Val Val Thr Val Ala Gly Val
 20 25 30
 Pro Leu Pro Asp Leu Ala Glu Glu Tyr Gly Thr Pro Leu Phe Val Val
 35 40 45
 Asp Glu Asp Asp Phe Arg Ser Arg Cys Arg Asp Met Ala Thr Ala Phe
 50 55 60

Gly Gly Pro Gly Asn Val His Tyr Ala Ser Lys Ala Phe Leu Thr Lys
 65 70 75 80
 Thr Ile Ala Arg Trp Val Asp Glu Glu Gly Leu Ala Leu Asp Ile Ala
 85 90 95
 Ser Ile Asn Glu Leu Gly Ile Ala Leu Ala Ala Gly Phe Pro Ala Ser
 100 105 110
 Arg Ile Thr Ala His Gly Asn Asn Lys Gly Val Glu Phe Leu Arg Ala
 115 120 125
 Leu Val Gln Asn Gly Val Gly His Val Val Leu Asp Ser Ala Gln Glu
 130 135 140
 Leu Glu Leu Leu Asp Tyr Val Ala Ala Gly Glu Gly Lys Ile Gln Asp
 145 150 155 160
 Val Leu Ile Arg Val Lys Pro Gly Ile Glu Ala His Thr His Glu Phe
 165 170 175
 Ile Ala Thr Ser His Glu Asp Gln Lys Phe Gly Phe Ser Leu Ala Ser
 180 185 190
 Gly Ser Ala Phe Glu Ala Ala Lys Ala Ala Asn Asn Ala Glu Asn Leu
 195 200 205
 Asn Leu Val Gly Leu His Cys His Val Gly Ser Gln Val Phe Asp Ala
 210 215 220
 Glu Gly Phe Lys Leu Ala Ala Glu Arg Val Leu Gly Leu Tyr Ser Gln
 225 230 235 240
 Ile His Ser Glu Leu Gly Val Ala Leu Pro Glu Leu Asp Leu Gly Gly
 245 250 255
 Gly Tyr Gly Ile Ala Tyr Thr Ala Ala Glu Glu Pro Leu Asn Val Ala
 260 265 270
 Glu Val Ala Ser Asp Leu Leu Thr Ala Val Gly Lys Met Ala Ala Glu
 275 280 285
 Leu Gly Ile Asp Ala Pro Thr Val Leu Val Glu Pro Gly Arg Ala Ile
 290 295 300
 Ala Gly Pro Ser Thr Val Thr Ile Tyr Glu Val Gly Thr Thr Lys Asp
 305 310 315 320
 Val His Val Asp Asp Lys Thr Arg Arg Tyr Ile Ala Val Asp Gly
 325 330 335
 Gly Met Ser Asp Asn Ile Arg Pro Ala Leu Tyr Gly Ser Glu Tyr Asp
 340 345 350
 Ala Arg Val Val Ser Arg Phe Ala Glu Gly Asp Pro Val Ser Thr Arg
 355 360 365
 Ile Val Gly Ser His Cys Glu Ser Gly Asp Ile Leu Ile Asn Asp Glu
 370 375 380
 Ile Tyr Pro Ser Asp Ile Thr Ser Gly Asp Phe Leu Ala Leu Ala Ala
 385 390 395 400
 Thr Gly Ala Tyr Cys Tyr Ala Met Ser Ser Arg Tyr Asn Ala Phe Thr
 405 410 415
 Arg Pro Ala Val Val Ser Val Arg Ala Gly Ser Ser Arg Leu Met Leu
 420 425 430
 Arg Arg Glu Thr Leu Asp Asp Ile Leu Ser Leu Glu Ala
 435 440 445

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA"

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:
AACCTCGTCA TGTTTGAGAA

20

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "synthetic DNA"

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:
CCGGCCTACA AAATCGTGCA

20

(2) INFORMATION FOR: SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1331 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Escherichia coli

(B) STRAIN: JM109

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 10..1197

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

AACCTCGTC ATG TTT GAG AAC ATT ACC GCC GCT CCT GCC GAC CCG ATT

48

Met Phe Glu Asn Ile Thr Ala Ala Pro Ala Asp Pro Ile

1

5

10

CTG GGC CTG GCC GAT CTG TTT CGT GCC GAT GAA CGT CCC GGC AAA ATT

96

Leu Gly Leu Ala Asp Leu Phe Arg Ala Asp Glu Arg Pro Gly Lys Ile

15

20

25

AAC CTC GGG ATT GGT GTC TAT AAA GAT GAG ACG GGC AAA ACC CCG GTA

144

Asn Leu Gly Ile Gly Val Tyr Lys Asp Glu Thr Gly Lys Thr Pro Val

CTG ACC AGC GTG AAA AAG GCT GAA CAG TAT CTG CTC GAA AAT GAA ACC

192

Leu Thr Ser Val Lys Lys Ala Glu Gln Tyr Leu Leu Glu Asn Glu Thr

50

55

60

ACC AAA AAT TAC CTC GGC ATT GAC GGC ATC CCT GAA TTT GGT CGC TGC

240

Thr Lys Asn Tyr Leu Gly Ile Asp Gly Ile Pro Glu Phe Gly Arg Cys

65

70

75

ACT CAG GAA CTG CTG TTT GGT AAA GGT AGC GCC CTG ATC AAT GAC AAA

288

Thr Gln Glu Leu Leu Phe Gly Lys Gly Ser Ala Leu Ile Asn Asp Lys

80

85

90

CGT GCT CGC ACG GCA CAG ACT CCG GGG GGC ACT GGC GCA CTA CGC GTG

336

Arg Ala Arg Thr Ala Gln Thr Pro Gly Gly Thr Gly Ala Leu Arg Val

GCT GCC GAT TTC CTG GCA AAA AAT ACC AGC GTT AAG CGT GTG TGG GTG

384

Ala Ala Asp Phe Leu Ala Lys Asn Thr Ser Val Lys Arg Val Trp Val

AGC AAC CCA AGC TGG CCG AAC CAT AAG AGC GTC TTT AAC TCT GCA GGT

432

Ser Asn Pro Ser Trp Pro Asn His Lys Ser Val Phe Asn Ser Ala Gly

130

135

140

CTG GAA GTT CGT GAA TAC GCT TAT TAT GAT GCG GAA AAT CAC ACT CTT

480

Leu Glu Val Arg Glu Tyr Ala Tyr Tyr Asp Ala Glu Asn His Thr Leu

145

150

155

5 GAC TTC GAT GCA CTG ATT AAC AGC CTG AAT GAA GCT CAG GCT GGC GAC 528
 Asp Phe Asp Ala Leu Ile Asn Ser Leu Asn Glu Ala Gln Ala Gly Asp
 160 165 170
 5 GTA GTG CTG TTC CAT GGC TGC TGC CAT AAC CCA ACC GGT ATC GAC CCT 576
 Val Val Leu Phe His Gly Cys His Asn Pro Thr Gly Ile Asp Pro
 175 180 185
 ACG CTG GAA CAA TGG CAA ACA CTG GCA CAA CTC TCC GTT GAG AAA GGC 624
 Thr Leu Glu Gln Trp Gln Thr Leu Ala Gln Leu Ser Val Glu Lys Gly
 190 195 200 205
 10 TGG TTA CCG CTG TTT GAC TTC GCT TAC CAG GGT TTT GCC CGT GGT CTG 672
 Trp Leu Pro Leu Phe Asp Phe Ala Tyr Gln Gly Phe Ala Arg Gly Leu
 210 215 220
 GAA GAA GAT GCT GAA GGA CTG CGC GCT TTC GCG GCT ATG CAT AAA GAG 720
 Glu Glu Asp Ala Glu Gly Leu Arg Ala Phe Ala Ala Met His Lys Glu
 225 230 235
 15 CTG ATT GTT GCC AGT TCC TAC TCT AAA AAC TTT GGC CTG TAC AAC GAG 768
 Leu Ile Val Ala Ser Ser Tyr Ser Lys Asn Phe Gly Leu Tyr Asn Glu
 240 245 250
 CGT GTT GGC GCT TGT ACT CTG GTT GCT GCC GAC AGT GAA ACC GTT GAT 816
 Arg Val Gly Ala Cys Thr Leu Val Ala Ala Asp Ser Glu Thr Val Asp
 255 260 265
 20 CGC GCA TTC AGC CAA ATG AAA GCG GCG ATT CGC GCT AAC TAC TCT AAC 864
 Arg Ala Phe Ser Gln Met Lys Ala Ala Ile Arg Ala Asn Tyr Ser Asn
 270 275 280 285
 CCA CCA GCA CAC GGC GCT TCT GTT GTT GCC ACC ATC CTG AGC AAC GAT 912
 Pro Pro Ala His Gly Ala Ser Val Val Ala Thr Ile Leu Ser Asn Asp
 290 295 300
 25 GCG TTA CGT GCG ATT TGG GAA CAA GAG CTG ACT GAT ATG CGC CAG CGT 960
 Ala Leu Arg Ala Ile Trp Glu Gln Glu Leu Thr Asp Met Arg Gln Arg
 305 310 315
 ATT CAG CGT ATG CGT CAG TTG TTC GTC AAT ACG CTG CAG GAA AAA GGC 1008
 Ile Gln Arg Met Arg Gln Leu Phe Val Asn Thr Leu Gln Glu Lys Gly
 320 325 330
 30 GCA AAC CGC GAC TTC AGC TTT ATC ATC AAA CAG AAC GGC ATG TTC TCC 1056
 Ala Asn Arg Asp Phe Ser Phe Ile Ile Lys Gln Asn Gly Met Phe Ser
 335 340 345
 TTC AGT GGC CTG ACA AAA GAA CAA GTG CTG CGT CTG CGC GAA GAG TTT 1104
 Phe Ser Gly Leu Thr Lys Glu Gln Val Leu Arg Leu Arg Glu Glu Phe
 350 355 360 365
 35 GGC GTA TAT GCG GTT GCT TCT GGT CGC GTA AAT GTG GCC GGG ATG ACA 1152
 Gly Val Tyr Ala Val Ala Ser Gly Arg Val Asn Val Ala Gly Met Thr
 370 375 380
 CCA GAT AAC ATG GCT CCG CTG TGC GAA GCG ATT GTG GCA GTG CTG 1197
 Pro Asp Asn Met Ala Pro Leu Cys Glu Ala Ile Val Ala Val Leu
 385 390 395
 TAAGCATTAA AAACAATGAA CGCGCTGAAA AGCGGGCTGA GACTGATGAC AAACGCAACA 1257
 TTGCCTGATG GCTACGCTTA TCAGGCCTAC GCGTCCCCTG CAATATTTTG AATTTGCACG 1317
 ATTTGTAGG CCGG 1331

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 396 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Met Phe Glu Asn Ile Thr Ala Ala Pro Ala Asp Pro Ile Leu Gly Leu

1

5

10

15

Ala Asp Leu Phe Arg Ala Asp Glu Arg Pro Gly Lys Ile Asn Leu Gly
 20 25 30
 Ile Gly Val Tyr Lys Asp Glu Thr Gly Lys Thr Pro Val Leu Thr Ser
 35 40 45
 Val Lys Lys Ala Glu Gln Tyr Leu Leu Glu Asn Glu Thr Thr Lys Asn
 50 55 60
 Tyr Leu Gly Ile Asp Gly Ile Pro Glu Phe Gly Arg Cys Thr Gln Glu
 65 70 75 80
 Leu Leu Phe Gly Lys Gly Ser Ala Leu Ile Asn Asp Lys Arg Ala Arg
 85 90 95
 Thr Ala Gln Thr Pro Gly Gly Thr Gly Ala Leu Arg Val Ala Ala Asp
 100 105 110
 Phe Leu Ala Lys Asn Thr Ser Val Lys Arg Val Trp Val Ser Asn Pro
 115 120 125
 Ser Trp Pro Asn His Lys Ser Val Phe Asn Ser Ala Gly Leu Glu Val
 130 135 140
 Arg Glu Tyr Ala Tyr Tyr Asp Ala Glu Asn His Thr Leu Asp Phe Asp
 145 150 155 160
 Ala Leu Ile Asn Ser Leu Asn Glu Ala Gln Ala Gly Asp Val Val Leu
 165 170 175
 Phe His Gly Cys Cys His Asn Pro Thr Gly Ile Asp Pro Thr Leu Glu
 180 185 190
 Gln Trp Gln Thr Leu Ala Gln Leu Ser Val Glu Lys Gly Trp Leu Pro
 195 200 205
 Leu Phe Asp Phe Ala Tyr Gln Gly Phe Ala Arg Gly Leu Glu Glu Asp
 210 215 220
 Ala Glu Gly Leu Arg Ala Phe Ala Ala Met His Lys Glu Leu Ile Val
 225 230 235 240
 Ala Ser Ser Tyr Ser Lys Asn Phe Gly Leu Tyr Asn Glu Arg Val Gly
 245 250 255
 Ala Cys Thr Leu Val Ala Ala Asp Ser Glu Thr Val Asp Arg Ala Phe
 260 265 270
 Ser Gln Met Lys Ala Ala Ile Arg Ala Asn Tyr Ser Asn Pro Pro Ala
 275 280 285
 His Gly Ala Ser Val Val Ala Thr Ile Leu Ser Asn Asp Ala Leu Arg
 290 295 300
 Ala Ile Trp Glu Gln Glu Leu Thr Asp Met Arg Gln Arg Ile Gln Arg
 305 310 315 320
 Met Arg Gln Leu Phe Val Asn Thr Leu Gln Glu Lys Gly Ala Asn Arg
 325 330 335
 Asp Phe Ser Phe Ile Ile Lys Gln Asn Gly Met Phe Ser Phe Ser Gly
 340 345 350
 Leu Thr Lys Glu Gln Val Leu Arg Leu Arg Glu Glu Phe Gly Val Tyr
 355 360 365
 Ala Val Ala Ser Gly Arg Val Asn Val Ala Gly Met Thr Pro Asp Asn
 370 375 380
 Met Ala Pro Leu Cys Glu Ala Ile Val Ala Val Leu
 385 390 395

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2517 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Brevibacterium lactofermentum*

(B) STRAIN: ATCC 13869

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

GATCAGCTTC	GGGTGTTGAA	GGGGCCGAAT	AAGGAACTCG	CGCAGTTGGG	TCGTAGTTTG	60
TTTAAACGAC	TTGGTGATGT	GTTGGCGTAT	TTCGATGTTG	GTGTCTCCAA	CGGTCCGGTC	120
GAAGCGATCA	ACGGACGGTT	GGAGCATTTG	CGTGGGATTG	CTCTAGGTTT	CCGTAAATTTG	180
AACCACTACA	TTCTGCGGTG	CCTTATCCAT	TCAGGGCAGT	TGGTCCATAA	GATCAATGCA	240
CTCTAAAACA	GGAAGAGCCC	CTTTACAAGC	GGCAAGACCA	AACTGGGTGA	CCGAAAATCT	300
TCAGGCCAAT	CAGTTTGTGT	CATATGGGAT	GGTTTTTAGA	CCTCGAAACC	ATCCCATATG	360
ACCGAAGCCC	GCGAAACTCT	GTGTTCTGTTG	GTGCGCTGGT	TCGGTCTCAA	TGCCTTGCCG	420
AAACCACAAC	GCCCCGAAAC	CCAAAACCTC	CCAATACATG	AAAAAACCAG	CTTCCCACCG	480
AAGTGAGAAG	CTGGTTTAGT	TTGCGGAGGA	TAGGGGATT	GAACCCCTGA	GGGATTGCTC	540
CCAACCCGCG	TTCCAGGCGA	GCGACATAGG	CCGCTAGTCG	AATCCTCCAG	CTAGAACGGC	600
TGCAACGCAT	GGTGCTTTG	TTCTGGGGAT	TAGATTACAC	AAAAGTCGTT	TAGAACTCA	660
AATCCGCTCG	CAGTTGGCGT	TTTCTGGGCG	GGTTCAGCTA	GAGTTATGCG	AAGGATCCCG	720
TGCGGCGTTT	ATCTTGTGAA	CTCCCCCAGG	GCAGGAATGC	AGCAAGGGTC	AGCGAGCTCT	780
GACGGGTGCG	CGGGGTCCCC	TAAACGTCT	AGAGTAGTGG	CTTGAGGTCA	CTGCTCTTTT	840
TTTGTGCCCT	TTTTTTGGTC	CGTCTATTTT	GCCACCACAT	GCGGAGGTAC	GCAGTTATGA	900
GTTCAAGTTT	GCTGCAGGAT	TTTGATGCAG	AGCGAATTGG	TCTGTTCCAC	GAGGACATTA	960
AACGCAAGTT	TGATGAGCTC	AAGTCAAAAA	ATCTGAAGCT	GGATCTTACT	CGCGGTAAGC	1020
CTTCGTCGGA	GCAGTTGGAT	TTGCTGATG	AGCTGTTGGC	GTTGCCTGGT	AAGGGCGATT	1080
TCAAGGCTGC	GGATGGTACT	GATGTCCGTA	ACTATGGCGG	GCTGGATGGC	ATTGTTGATA	1140
TTCGTCAGAT	TTGGGCGGAT	TTGCTGGGTG	TTCTGTGGA	GCAGGTGCTG	GCGGGGGATG	1200
CTTCGAGCTT	GAACATCATG	TTTGATGTGA	TCAGCTGGTC	GTACATTTTT	GGTAACAATG	1260
ATTCGGTTCA	GCCTTGGTCTG	AAGGAAGAAA	CTGTTAAGTG	GATTTGTCCT	GTTCCGGGAT	1320
ATGATCGCCA	TTTCTCCATC	ACGGAGCGTT	TCGGCTTTGA	GATGATTCT	GTGCCAATGA	1380
ATGAAGACGG	CCCTGATATG	GATGCTGTTG	AGGAATTGGT	CAAGGATCCG	CAGGTTAAGG	1440
GCATGTGGGT	TGTGCCGGTA	TTTTCTAACC	CGACTGGTTT	CACGGTGTCG	GAGGACGTCG	1500
CAAAGCGTCT	GAGCACGATG	GAAACTGCCG	CGCCGGAATT	CCGCGTGGTG	TGGGATAACG	1560
CTTACGCCGT	TCATACTCTG	ACCGATGAGT	TCCCTGAGGT	CATCGACATC	GTTGGGCTTG	1620
GTGAGGCGGC	GGGTAACCCG	AACCGTTTCT	GGGCGTTCAC	TTCTACTTCG	AAGATCACTC	1680
TCGCGGGTGC	GGGCGTGTCC	TTCTTCATGA	CTTCTGCGGA	GAACCGTAAG	TGGTACTCCG	1740
GTGATGCGGG	TATCCGTGGC	ATTGGCCCTA	ACAAGGTCAA	TCAGTTGGCT	CATGCGCGTT	1800
ACTTTGGCGA	TGCTGAGGGA	GTGCGCGCGG	TGATGCGTAA	GCATGCTGCG	TCGTTGGCTC	1860
CGAAGTTCAA	CAAGGTTCTG	GAGATCCTGG	ATTCTCGCCT	TGCTGAGTAC	GGTGTGCGGC	1920
AGTGGAAGT	CCCTGCGGGC	GGTACTTCA	TTTCCCTTGA	TGTGGTTCCT	GGTACGGCAT	1980
CTCGTGTGGC	TGAGTTGGCT	AAGGAAGCCG	GCATTGCGTT	GACGGGTGCG	GGTTCTTCTT	2040
ACCCGCTGCG	TCAGGATCCG	GAGAACAAGA	ACCTCCGTTT	GGCGCCTTCT	CTGCCTCCTG	2100
TTGAGGAACT	TGAGGTTGCC	ATGGATGGCG	TGGCTACGTG	TGTTTTGCTG	GCAGCTGCGG	2160
AGCACTACGC	TAGCTAGAGT	GAATACCGCG	GAAACTGCAC	ATTGGATTAA	CCGTTTGCTG	2220
CCGGGTCAGC	CGGAGTTTCA	CCAGGTTGGC	CGGTTTAAAG	TGGCGGGTTA	CACGCTTGAT	2280
GATGAGTCAA	TTGCGTGTTC	TGTCAATTTT	GGGCGCGTCA	ACACGGGCCT	GGTCACCGAG	2340
ACAGGCGCGG	AAACCGTCGA	TGTGCGAAGT	GAGATTTTGA	GCCTGGCCAG	GGCCGACGTG	2400
TCCGTGCGCT	GGCGCGCCGT	CGGCGCTGCT	GCAACAATGC	TTCTCGACGC	CTCCCTCTCC	2460
TTCAAATCCG	GCACCGATTC	CAGTGTCACT	CCCATGCATG	CCCAACCGGG	ACAGATC	2517

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 bases

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "synthetic DNA"

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

GATCAACGGA CGGTTGGAGC ATT

23

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 bases
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "synthetic DNA"
 (iv) ANTI-SENSE: yes
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:
 GGTATTCACT CTAGCTAGCG TAG 23

(2) INFORMATION FOR SEQ ID NO:28:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 23 bases
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "synthetic DNA"
 (iv) ANTI-SENSE: no
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:
 GAGCTCAAGT CAAAAAATCT GAA 23

(2) INFORMATION FOR SEQ ID NO:29:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 23 bases
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "synthetic DNA"
 (iv) ANTI-SENSE: yes
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:
 GATCTGTCCC GGTGGGCAT GCA 23

(2) INFORMATION FOR SEQ ID NO:30:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 2517 bases
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: genomic DNA
 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Brevibacterium lactofermentum
 (B) STRAIN: ATCC 13869
 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 879..2174
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:
 GATCAGCTTC GGGTGTGAA GGGGCCGAAT AAGGAACTCG CGCAGTTGGG TCGTAGTTTG 60
 TTTAAACGAC TTGGTGATGT GTTGGCGTAT TTCGATGTTG GTGTCTCCAA CGGTCCGGTC 120
 GAAGCGATCA ACGGACGGTT GGAGCATTTG CGTGGGATTG CTCTAGGTTT CCGTAATTTG 180
 AACCCTACA TTCTGCGGTG CCTTATCCAT TCAGGGCAGT TGGTCCATAA GATCAATGCA 240
 CTCTAAAACA GGAAGAGCCC CTTACAAGC GGCAAGACCA AACTGGGTGA CCGAAAATCT 300
 TCAGGCCAAT CAGTTTGGT CATATGGGAT GGTTTTGA CCGGAAACC ATCCCATATG 360
 ACCGAAGCCC GCGAAACTCT GTGTTGTTG GTGCGCTGGT TCGGTCTCAA TGCCTTGCCG 420
 AAACCACAAC GCCCCGAAAC CCAAACTCC CCAATACATG AAAAAACCAG CTTCCCACCG 480
 AAGTGAGAAG CTGGTTTAGT TTGCGGAGGA TAGGGGATTG GAACCCCTGA GGGATTGCTC 540
 CCAACCCGCG TTCCAGGCGA GCGACATAGG CCGTAGTCG AATCCTCCAG CTAGAACGGC 600
 TGCAACGCAT GGCTGCTTGT TTCTGGGGAT TAGATTACAC AAAAGTCGTT TAGAACTCA 660

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	Thr	Ser	Ala	Glu	Asn	Arg	Lys	Trp	Tyr	Ser	Gly	His	Ala	Gly	Ile	Arg	
			280					285					290				
5	GGC	ATT	GGC	CCT	AAC	AAG	GTC	AAT	CAG	TTG	GCT	CAT	GCG	CGT	TAC	TTT	1805
	Gly	Ile	Gly	Pro	Asn	Lys	Val	Asn	Gln	Leu	Ala	His	Ala	Arg	Tyr	Phe	
		295				300					305						
	GGC	GAT	GCT	GAG	GGA	GTG	CGC	GCG	GTG	ATG	CGT	AAG	CAT	GCT	GCG	TCG	1853
	Gly	Asp	Ala	Glu	Gly	Val	Arg	Ala	Val	Met	Arg	Lys	His	Ala	Ala	Ser	
		310			315						320				325		
10	TTG	GCT	CCG	AAG	TTC	AAC	AAG	GTT	CTG	GAG	ATC	CTG	GAT	TCT	CGC	CTT	1901
	Leu	Ala	Pro	Lys	Phe	Asn	Lys	Val	Leu	Glu	Ile	Leu	Asp	Ser	Arg	Leu	
				330						335					340		
	GCT	GAG	TAC	GGT	GTC	GCG	CAG	TGG	ACT	GTC	CCT	GCG	GGC	GGT	TAC	TTC	1949
	Ala	Glu	Tyr	Gly	Val	Ala	Gln	Trp	Thr	Val	Pro	Ala	Gly	Gly	Tyr	Phe	
			345					350					355				
15	ATT	TCC	CTT	GAT	GTG	GTT	CCT	GGT	ACG	GCA	TCT	CGT	GTG	GCT	GAG	TTG	1997
	Ile	Ser	Leu	Asp	Val	Val	Pro	Gly	Thr	Ala	Ser	Arg	Val	Ala	Glu	Leu	
			360				365					370					
	GCT	AAG	GAA	GCC	GGC	ATT	GCG	TTG	ACG	GGT	GCG	GGT	TCT	TCT	TAC	CCG	2045
	Ala	Lys	Glu	Ala	Gly	Ile	Ala	Leu	Thr	Gly	Ala	Gly	Ser	Ser	Tyr	Pro	
		375				380					385						
20	CTG	CGT	CAG	GAT	CCG	GAG	AAC	AAG	AAC	CTC	CGT	TTG	GCG	CCT	TCT	CTG	2093
	Leu	Arg	Gln	Asp	Pro	Glu	Asn	Lys	Asn	Leu	Arg	Leu	Ala	Pro	Ser	Leu	
		390				395					400				405		
	CCT	CCT	GTT	GAG	GAA	CTT	GAG	GTT	GCC	ATG	GAT	GGC	GTG	GCT	ACG	TGT	2141
	Pro	Pro	Val	Glu	Glu	Leu	Glu	Val	Ala	Met	Asp	Gly	Val	Ala	Thr	Cys	
				410				415					420				
25	GTT	TTG	CTG	GCA	GCT	GCG	GAG	CAC	TAC	GCT	AGC	TAGAGTGAAT	ACCGCGGAAA				2194
	Val	Leu	Leu	Ala	Ala	Ala	Glu	His	Tyr	Ala	Ser						
				425				430									
	CTGCACATTG	GATTAACCGT	TTGCTGCCGG	GTCAGCCGGA	GTTTCACCAG	GTGGCGCGT											2254
	TTAAAGTGGC	GGGTACACG	CTTGATGATG	AGTCAATTGC	GTGTCTGTC	AATTCGGGC											2314
	GCGTCAACAC	GGGCTGGTC	ACCGAGACAG	GCGCGGAAC	CGTCGATGTG	CGAAGTGAGA											2374
30	TTTGAGCCT	GGCCAGGGCC	GACGTGTCCG	TGCCTGGGCG	CGCCGTCGGC	GCTGCTGCAA											2434
	CAATGCTTCT	CGACGCCTCC	CTCTCCTTCA	AATCCGCCAC	CGATTCCAGT	GTCACTCCCA											2494
	TGCATGCCCA	ACCGGGACAG	ATC														2517

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 432 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

40	Met	Arg	Arg	Tyr	Ala	Val	Met	Ser	Ser	Val	Ser	Leu	Gln	Asp	Phe	Asp
	1				5					10					15	
	Ala	Glu	Arg	Ile	Gly	Leu	Phe	His	Glu	Asp	Ile	Lys	Arg	Lys	Phe	Asp
			20					25						30		
	Glu	Leu	Lys	Ser	Lys	Asn	Leu	Lys	Leu	Asp	Leu	Thr	Arg	Gly	Lys	Pro
			35				40						45			
45	Ser	Ser	Glu	Gln	Leu	Asp	Phe	Ala	Asp	Glu	Leu	Leu	Ala	Leu	Pro	Gly
		50				55						60				
	Lys	Gly	Asp	Phe	Lys	Ala	Ala	Asp	Gly	Thr	Asp	Val	Arg	Asn	Tyr	Gly
		65			70					75					80	
	Gly	Leu	Asp	Gly	Ile	Val	Asp	Ile	Arg	Gln	Ile	Trp	Ala	Asp	Leu	Leu
				85					90						95	
50	Gly	Val	Pro	Val	Glu	Gln	Val	Leu	Ala	Gly	Asp	Ala	Ser	Ser	Leu	Asn
			100					105					110			
	Ile	Met	Phe	Asp	Val	Ile	Ser	Trp	Ser	Tyr	Ile	Phe	Gly	Asn	Asn	Asp

115 120 125
 Ser Val Gln Pro Trp Ser Lys Glu Glu Thr Val Lys Trp Ile Cys Pro
 130 135 140
 5 Val Pro Gly Tyr Asp Arg His Phe Ser Ile Thr Glu Arg Phe Gly Phe
 145 150 155 160
 Glu Met Ile Ser Val Pro Met Asn Glu Asp Gly Pro Asp Met Asp Ala
 165 170 175
 Val Glu Glu Leu Val Lys Asp Pro Gln Val Lys Gly Met Trp Val Val
 180 185 190
 10 Pro Val Phe Ser Asn Pro Thr Gly Phe Thr Val Ser Glu Asp Val Ala
 195 200 205
 Lys Arg Leu Ser Thr Met Glu Thr Ala Ala Pro Asp Phe Arg Val Val
 210 215 220
 Trp Asp Asn Ala Tyr Ala Val His Thr Leu Thr Asp Glu Phe Pro Glu
 225 230 235 240
 15 Val Ile Asp Ile Val Gly Leu Gly Glu Ala Ala Gly Asn Pro Asn Arg
 245 250 255
 Phe Trp Ala Phe Thr Ser Thr Ser Lys Ile Thr Leu Ala Gly Ala Gly
 260 265 270
 20 Val Ser Phe Phe Met Thr Ser Ala Glu Asn Arg Lys Trp Tyr Ser Gly
 275 280 285
 His Ala Gly Ile Arg Gly Ile Gly Pro Asn Lys Val Asn Gln Leu Ala
 290 295 300
 His Ala Arg Tyr Phe Gly Asp Ala Glu Gly Val Arg Ala Val Met Arg
 305 310 315 320
 25 Lys His Ala Ala Ser Leu Ala Pro Lys Phe Asn Lys Val Leu Glu Ile
 325 330 335
 Leu Asp Ser Arg Leu Ala Glu Tyr Gly Val Ala Gln Trp Thr Val Pro
 340 345 350
 Ala Gly Gly Tyr Phe Ile Ser Leu Asp Val Val Pro Gly Thr Ala Ser
 355 360 365
 30 Arg Val Ala Glu Leu Ala Lys Glu Ala Gly Ile Ala Leu Thr Gly Ala
 370 375 380
 Gly Ser Ser Tyr Pro Leu Arg Gln Asp Pro Glu Asn Lys Asn Leu Arg
 385 390 395 400
 35 Leu Ala Pro Ser Leu Pro Pro Val Glu Glu Leu Glu Val Ala Met Asp
 405 410 415
 Gly Val Ala Thr Cys Val Leu Leu Ala Ala Ala Glu His Tyr Ala Ser
 420 425 430

Claims

- 45 1. A recombinant DNA autonomously replicable in cells of coryneform bacteria, comprising a DNA sequence coding for an aspartokinase in which feedback inhibition by L-lysine and L-threonine is substantially desensitized, a DNA sequence coding for a dihydrodipicolinate reductase, a DNA sequence coding for dihydrodipicolinate synthase, a DNA sequence coding for diaminopimelate decarboxylase, and a DNA sequence coding for aspartate aminotransferase.
- 50 2. The recombinant DNA according to claim 1, wherein said aspartokinase in which feedback inhibition by L-lysine and L-threonine is substantially desensitized is an aspartokinase originating from coryneform bacteria, and wherein said aspartokinase is a mutant aspartokinase in which an amino acid residue corresponding to a 279th alanine residue as counted from its N-terminal in the amino acid sequence shown in SEQ ID NO: 5 is changed into an amino acid residue other than alanine and other than acidic amino acid in its α -subunit, and an amino acid residue corresponding to a 30th alanine residue as counted from its N-terminal in the amino acid sequence shown in SEQ ID NO: 7 is changed into an amino acid residue other than alanine and other than acidic amino acid in its β -subunit.
- 55

3. The recombinant DNA according to claim 1, wherein said DNA sequence coding for the dihydropicolinate reductase codes for an amino acid sequence shown in SEQ ID NO: 15, or an amino acid sequence substantially the same as the amino acid sequence shown in SEQ ID NO: 15.
- 5 4. The recombinant DNA according to claim 1, wherein said DNA sequence coding for the dihydropicolinate synthase codes for an amino acid sequence shown in SEQ ID NO: 11, or an amino acid sequence substantially the same as the amino acid sequence shown in SEQ ID NO: 11.
- 10 5. The recombinant DNA according to claim 1, wherein said DNA sequence coding for the diaminopimelate decarboxylase codes for an amino acid sequence shown in SEQ ID NO: 19, or an amino acid sequence substantially the same as the amino acid sequence shown in SEQ ID NO: 19.
- 15 6. The recombinant DNA according to claim 1, wherein said DNA sequence coding for the aspartate aminotransferase codes for an amino acid sequence shown in SEQ ID NO: 24 or 31, or an amino acid sequence substantially the same as the amino acid sequence shown in SEQ ID NO: 24 or 31.
- 20 7. A coryneform bacterium harboring an aspartokinase in which feedback inhibition by L-lysine and L-threonine is substantially desensitized, and comprising an enhanced DNA sequence coding for a dihydropicolinate reductase, an enhanced DNA sequence coding for dihydropicolinate synthase, an enhanced DNA sequence coding for diaminopimelate decarboxylase and an enhanced DNA sequence coding for aspartate aminotransferase.
- 25 8. The coryneform bacterium according to claim 7, transformed by introduction of the recombinant DNA as defined in claim 1.
9. A method for producing L-lysine comprising the steps of cultivating said coryneform bacterium as defined in claim 8 in an appropriate medium to allow L-lysine to be produced and accumulated in a culture of the bacterium, and collecting L-lysine from the culture.
- 30 10. A DNA coding for a protein comprising an amino acid sequence shown in SEQ ID NO: 31.
11. The DNA according to claim 10, which comprises a nucleotide sequence of nucleotide number 879 to 2174 in a nucleotide sequence shown in SEQ ID NO: 30.
- 35 12. A vector pVK7, which is autonomously replicable in cells of Escherichia coli and Brevibacterium lactofermentum, and comprising a multiple cloning site and lacZ.

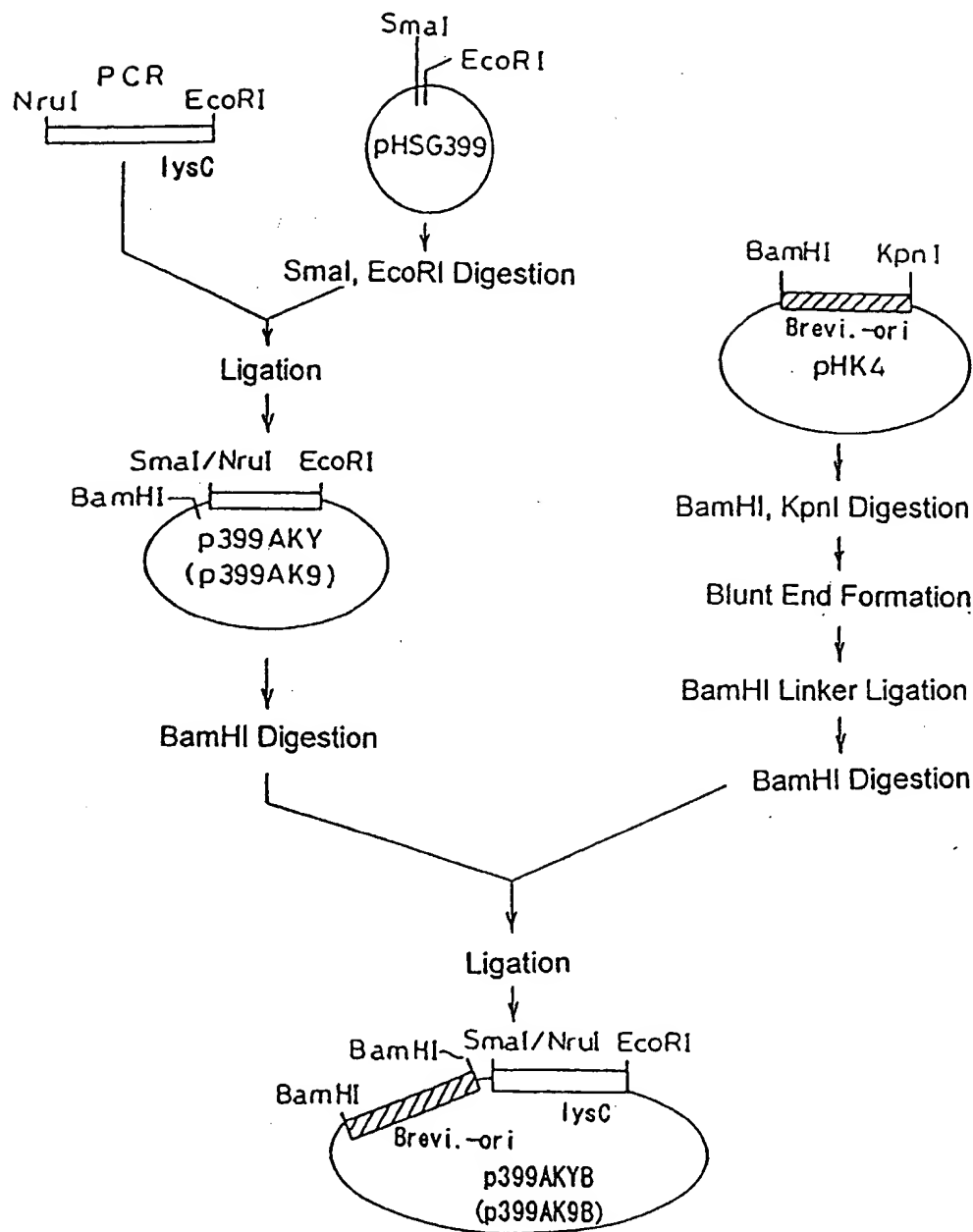


FIG. 1

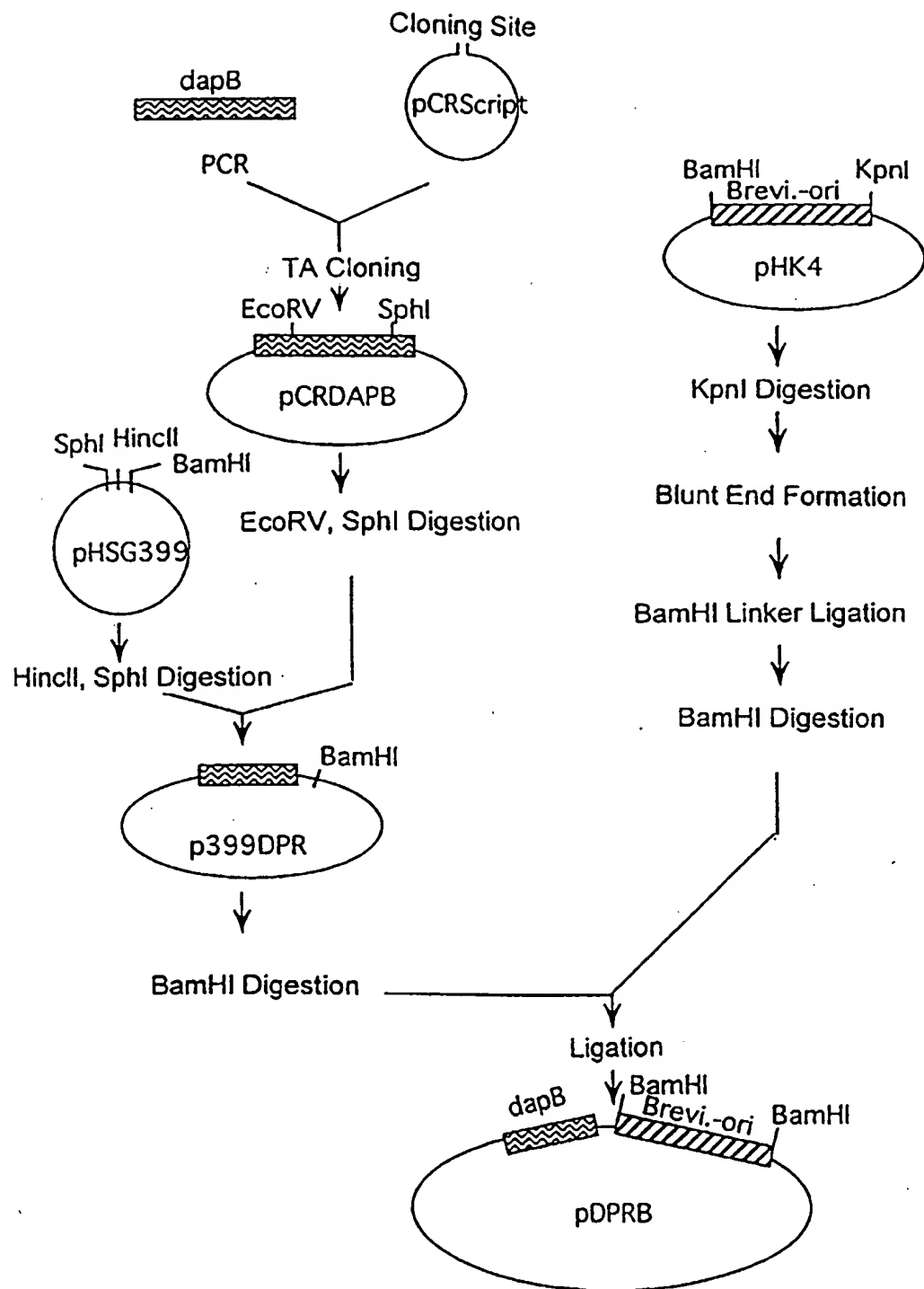


FIG. 2

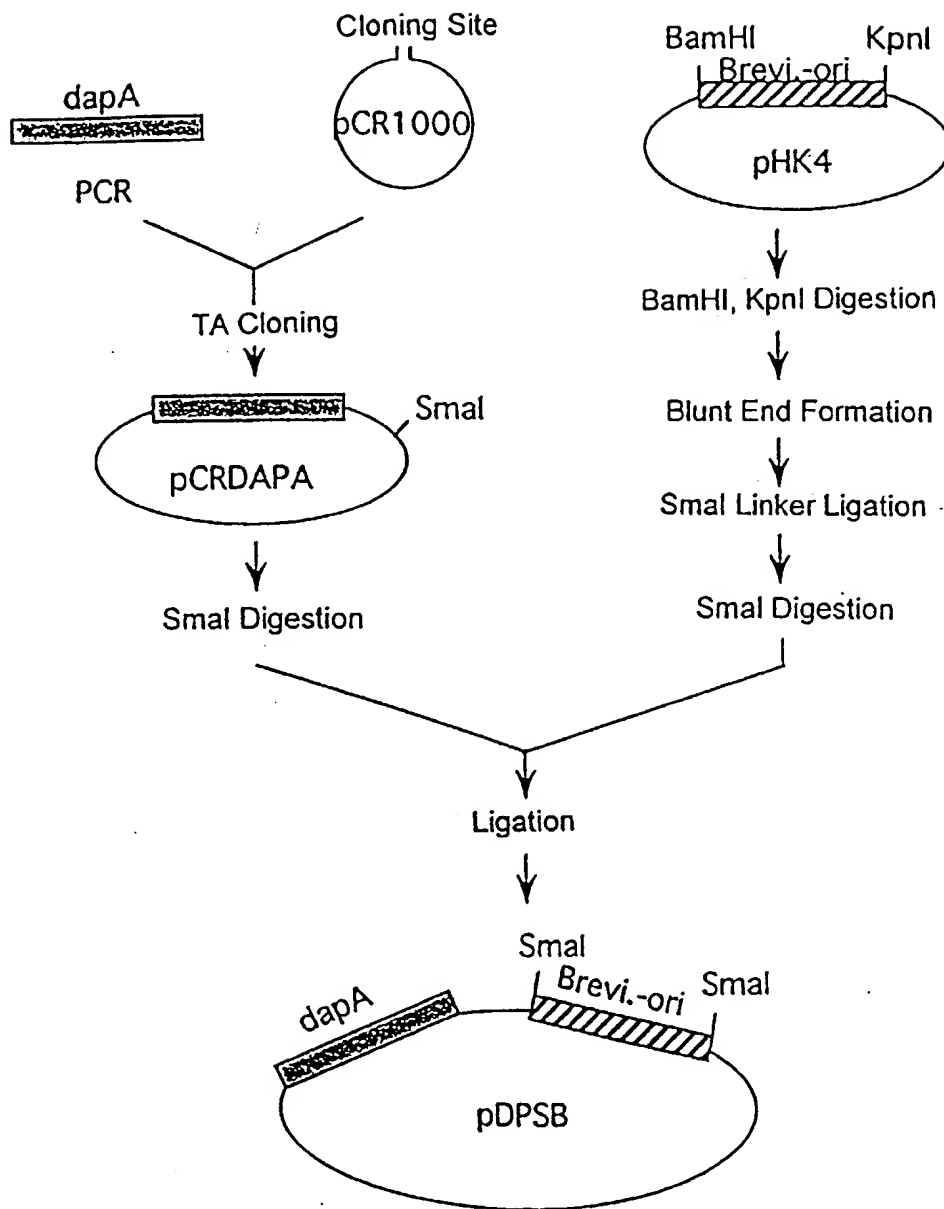


FIG. 3

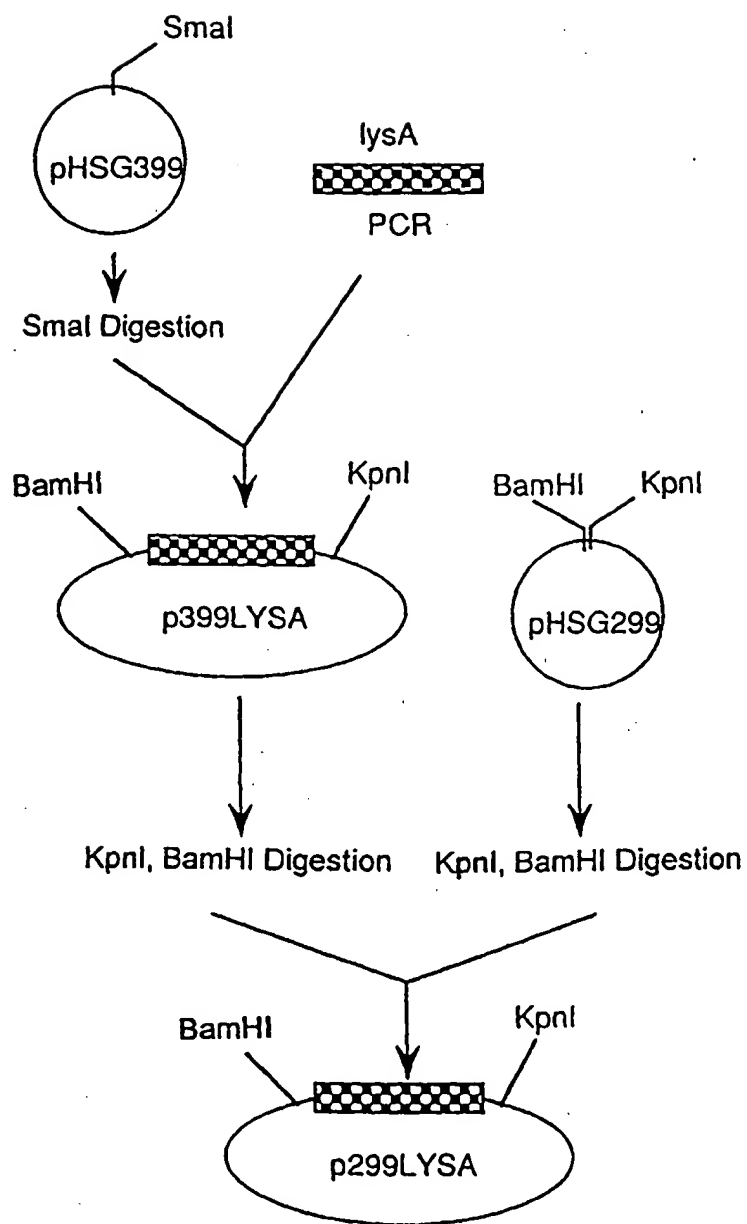


FIG. 4

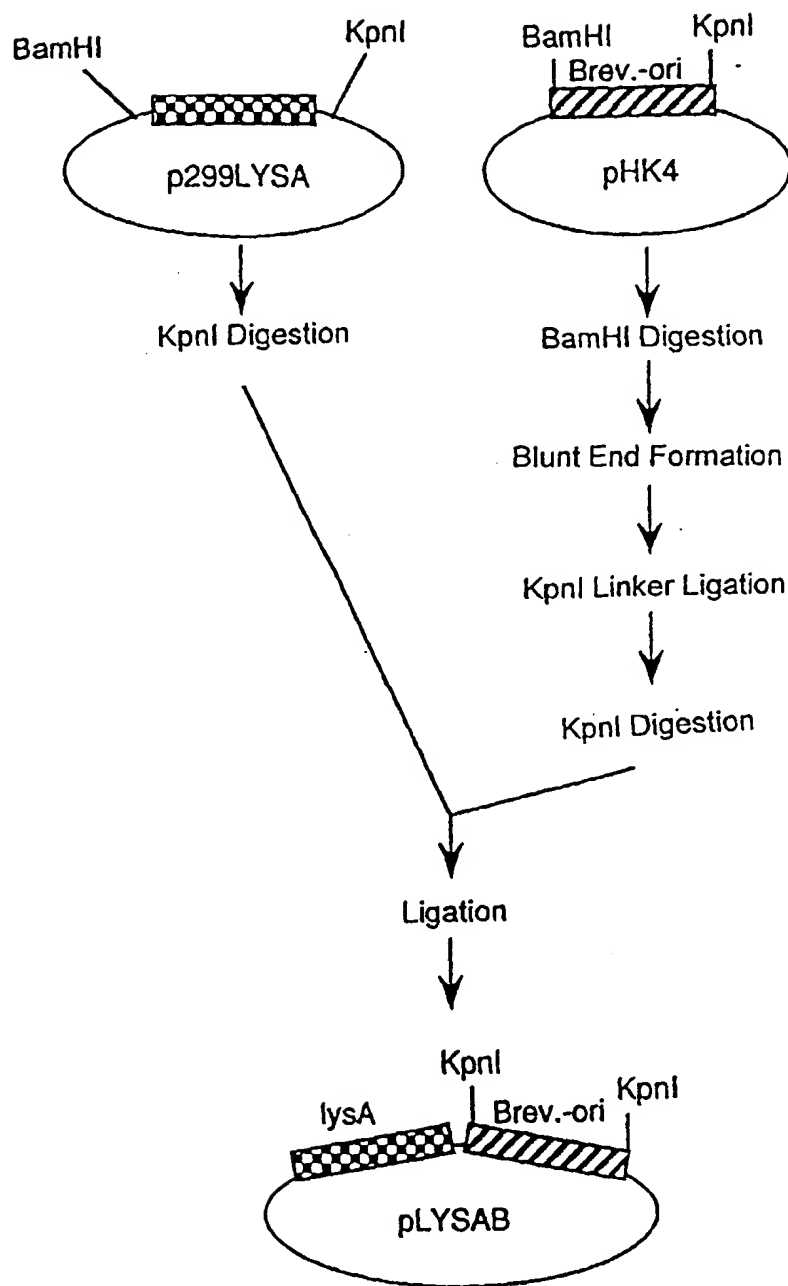


FIG. 5

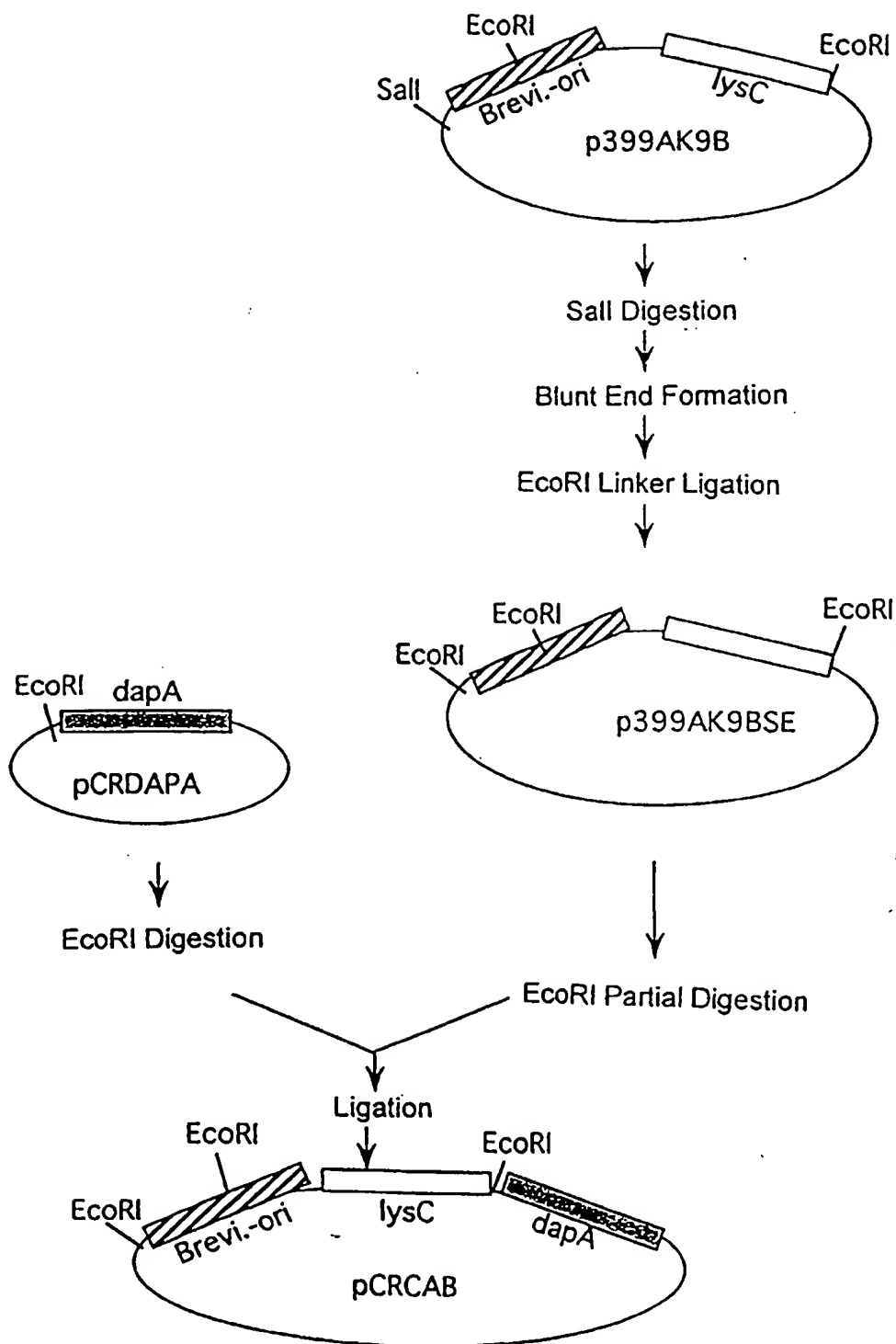


FIG. 6

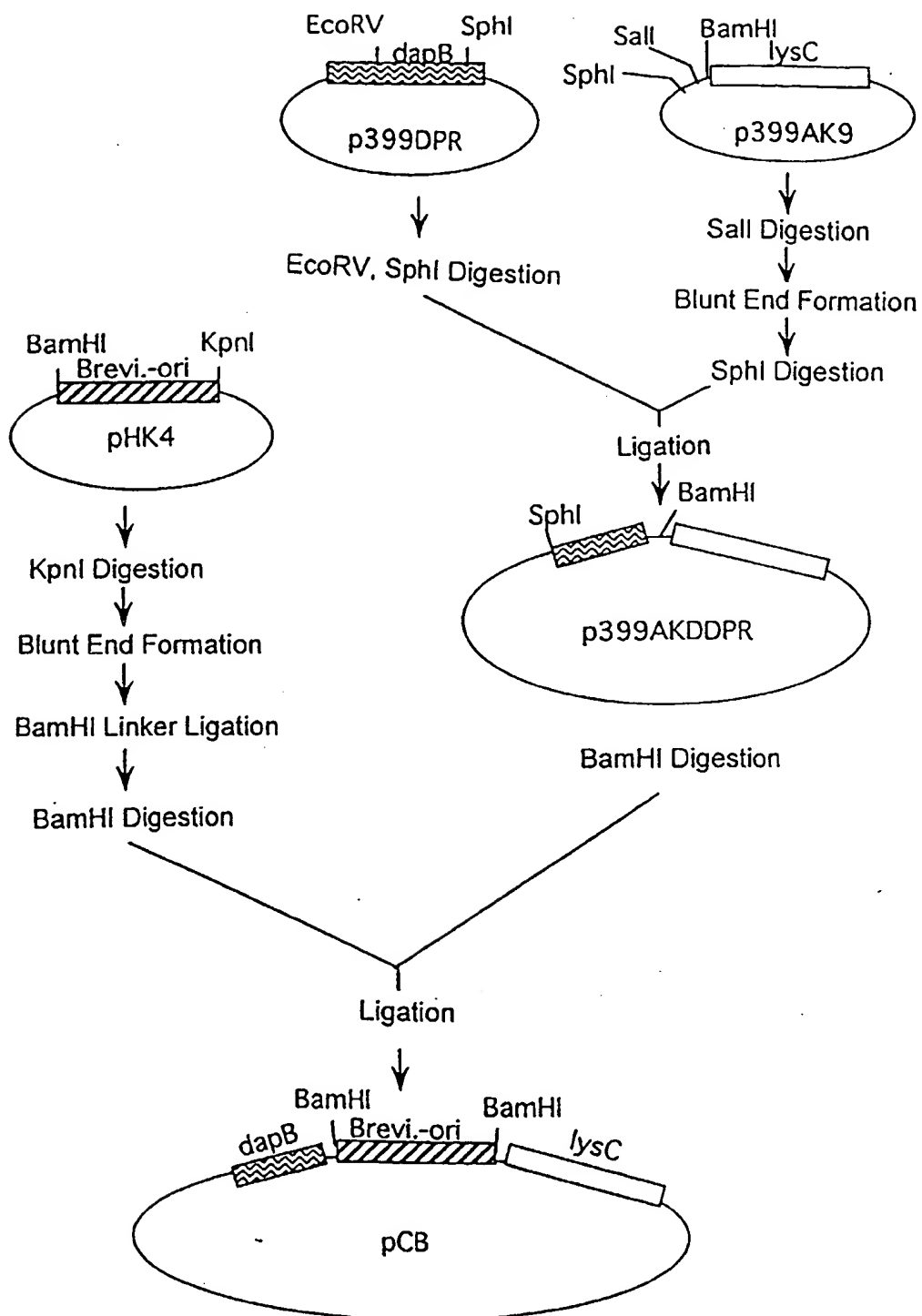


FIG. 7

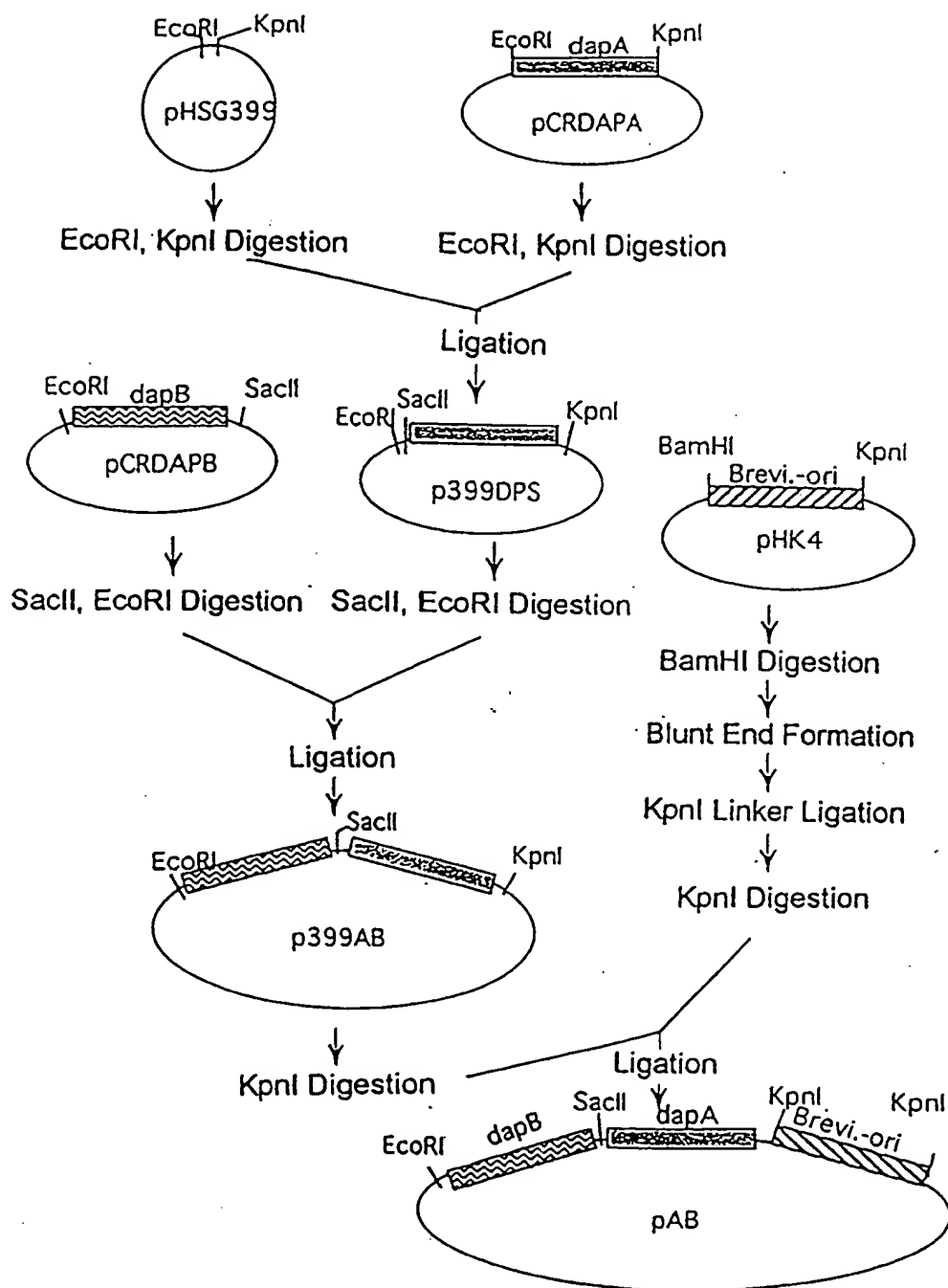


FIG. 8

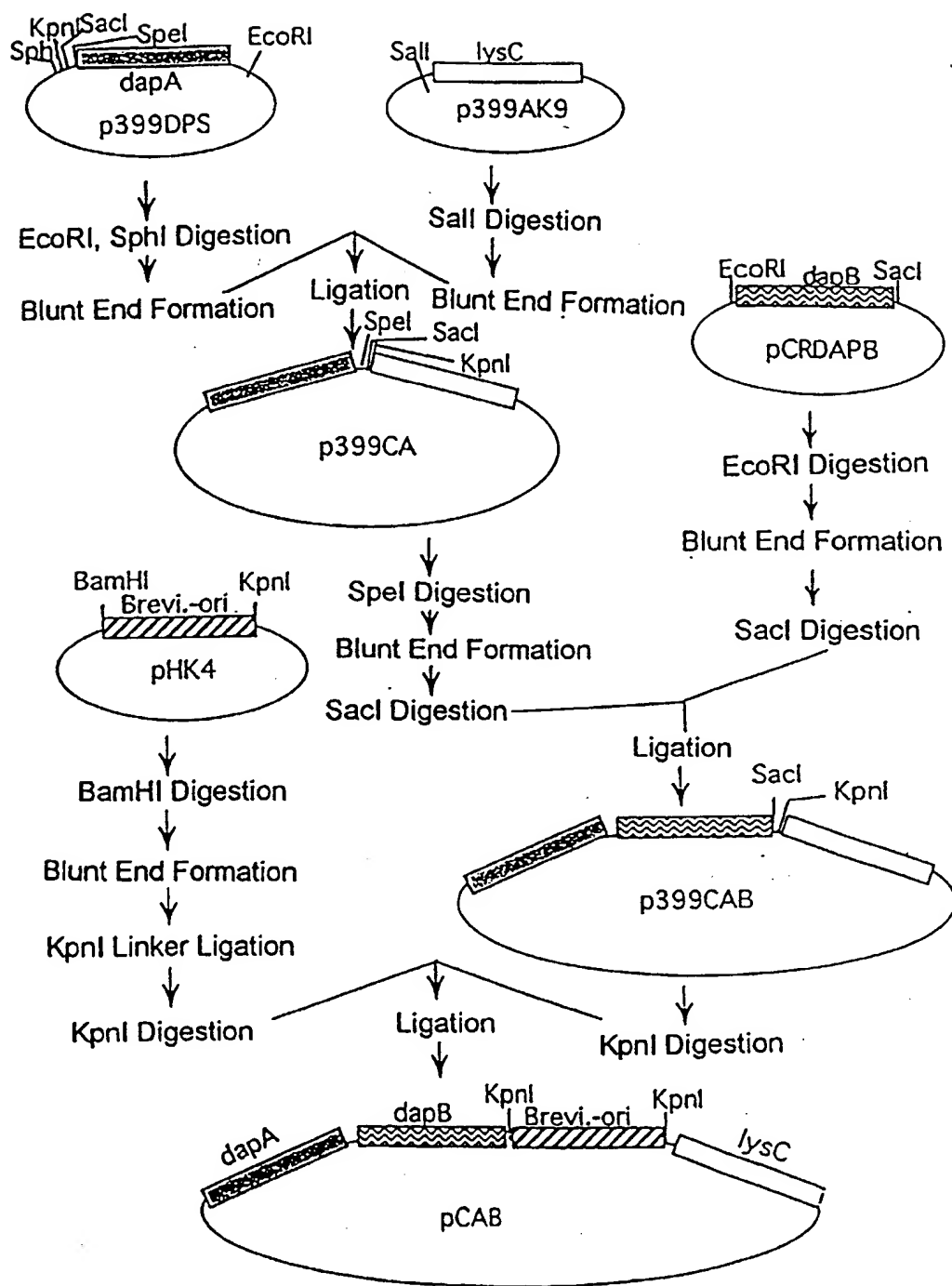


FIG. 9

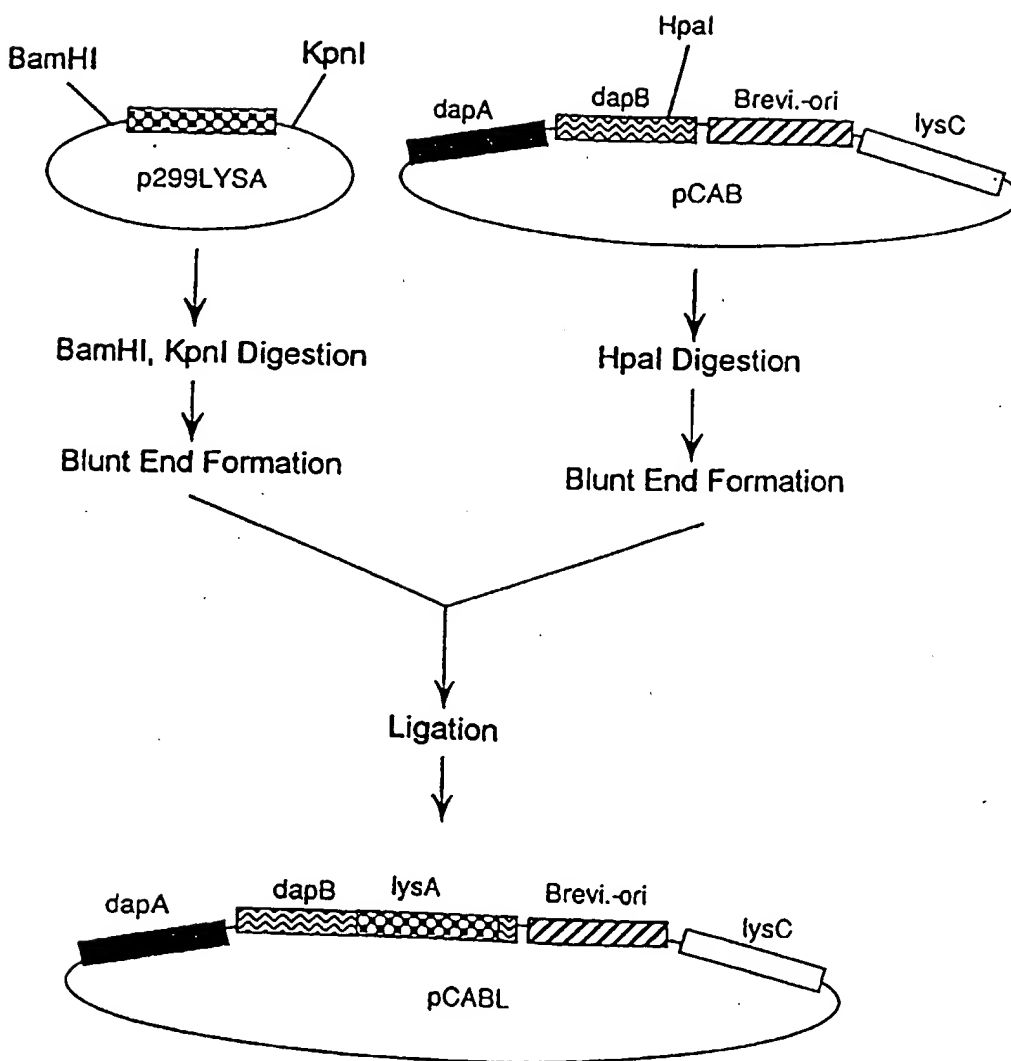


FIG. 10

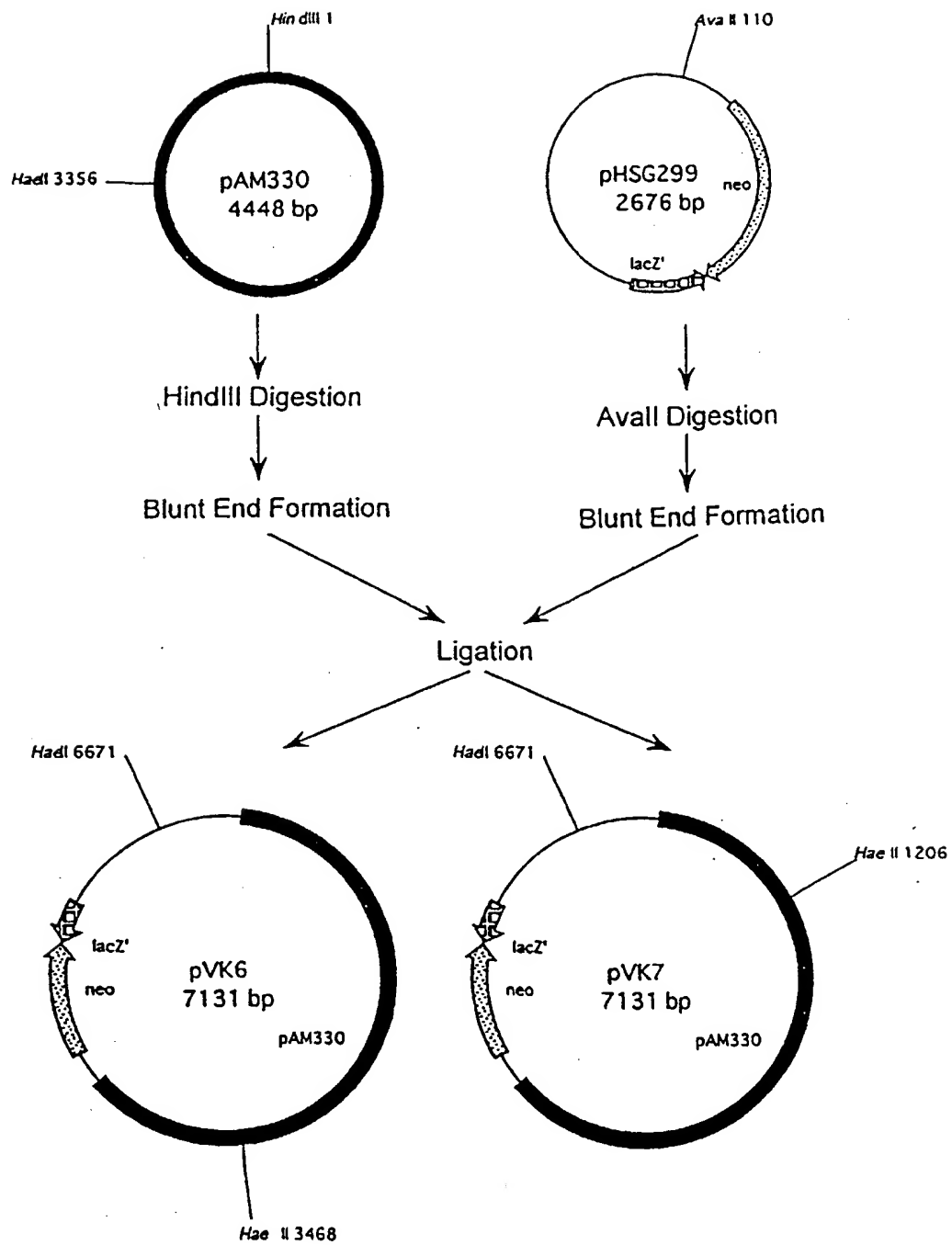


FIG. 11

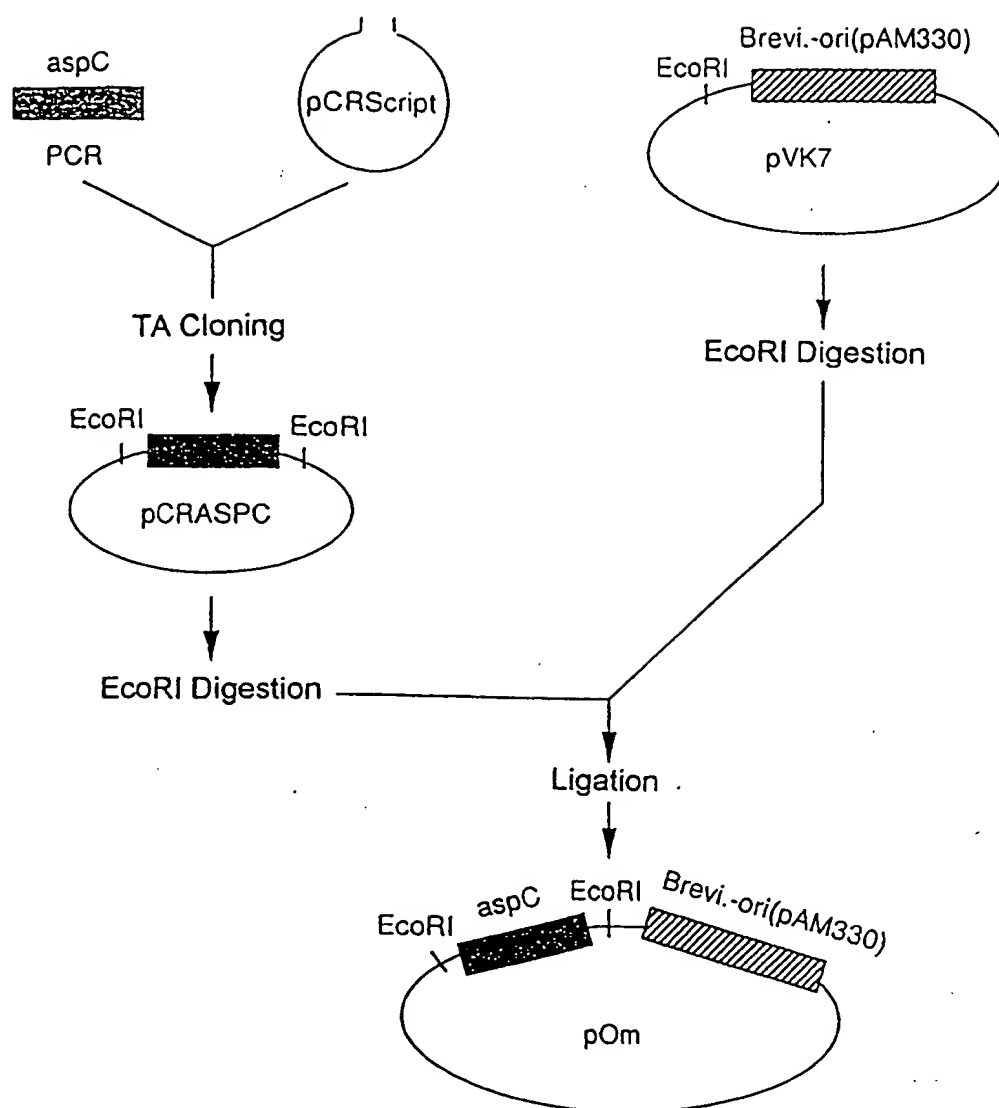


FIG. 12

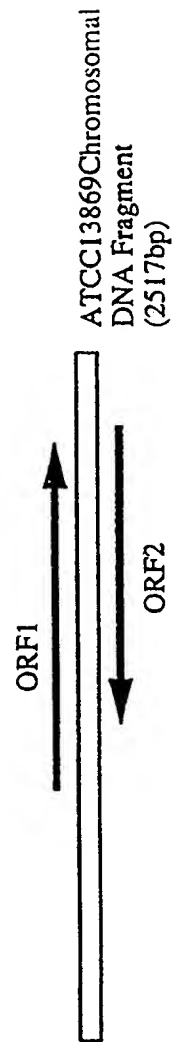


FIG. 13

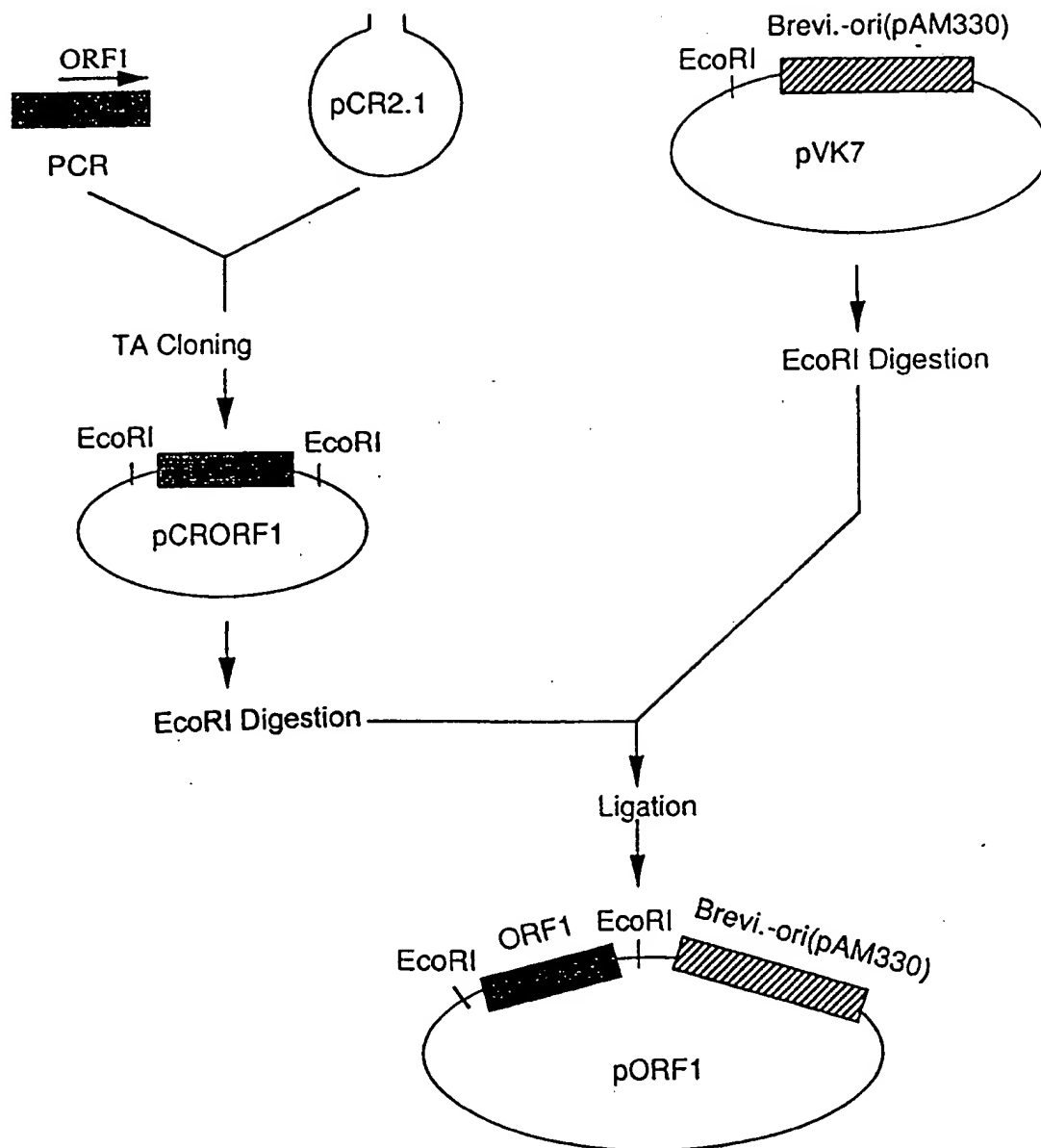


FIG. 14

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